# *ADVANCED RADIATION THERAPEUTICS - RADIATION INJURY MITIGATION*

# WORKSHOP

**January 25, 2010**

***Jointly Sponsored by***

**THE NATIONAL CANCER INSTITUTE**

**and**

**THE NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASE**

**Executive Plaza Conference Center**

**Rockville, Maryland**

**The National Institutes of Health**

# *ADVANCED RADIATION THERAPEUTICS - RADIATION INJURY MITIGATION*

## AGENDA

8:00 -8:15 am: Welcome

8:15-9:45 am: Panel 1: The clinical problem (Moderator: Andy Trotti)

What are the most serious toxicities from radiation therapy? (Bhadrasain Vikram 15 min/Andy Trotti 15 min)
What are the mechanisms of these and other common RT toxicities? (Mitch Anscher 15 min)
Patient advocate perspective (David Klein 20 min)
Discussion

9:45-11:00 am: Panel 2: FDA issues (Moderator: Paul Okunieff)

Patient Reported Outcomes (Ethan Basch 15 min)
DoD Perspective (TBA 15 min)
Cancer clinical trials aimed at decreasing toxicity (Gary Morrow 15 min)
Discussion

11:00-11:15: Break

11:15am-1:15 pm: Panel 3: What are the most promising drugs in the pipeline? (Moderator: Walter Curran)
Overview (Ian Stratford 20 min)
CMCR speakers (Drs. Okunieff, Moulder, Hauer-Jensen, Georges and Chao: 10 min each)
Discussion (to include industry representatives)

1:15-2:15 pm: Lunch

2:15-3:45 pm: Panel 4: Generating preclinical/biomarker data for clinical trials (Moderator: Steve Brown)
Present the NCI document and challenges re: clinical trials (Julie Ryan 15 min)
Preclinical studies showing protection of normal tissues and lack of protection
for tumors (Steve Brown, Adam Dicker: 10 min each)
Phase "zero" vs. phase I designs (Anthony Murgo 15 min)
Discussion

3:45-4:00: Break

4:00-5:30 pm: Panel 5: Designing and conducting clinical trials (Moderator: Lisa Kachnic)

Lessons from RTOG 9801 (re "disconnect" and radiation protection) (Ben Movsas 15 min)
Designing phase II or III clinical trials to demonstrate RT mitigation (Deb Bruner 15 min)
Discussion (to include co-op group disease site chairs: Drs. Curran, Choy, Dicker, and Mehta)

5:30-6:00 pm: Summary/Next steps

# PANEL 1

# WHAT ARE THE MOST SERIOUS ADVERSE EVENTS DURING AND AFTER RADIATION THERAPY?

*Bhadrasain Vikram, MD*
*Chief-Clinical Radiation Oncology Branch*
*National Cancer Institute*
*vikramb@mail.nih.gov*

Serious adverse events frequently occur among patients with many kinds of common cancers during and after radiation therapy or radiochemotherapy. The impact of advanced technologies (IMRT, Protons, etc.) in that regard has until now been rather modest. Substantial room for improvement remains with regard to both short-term and long-term adverse effects resulting from injuries to:

- the alimentary tract, from the mouth to the anus
- the skin
- the bladder and the urethra
- the lungs
- the brain
- the liver
- the kidneys

The serious adverse events reported in some recent landmark publications are summarized on the attached tables, reproduced from:

**Vikram B, Coleman CN, Deye JA. Current status and future potential of advanced technologies in radiation oncology. Part 2. State of the science by anatomic site. Oncology (Williston Park). 2009 Apr 15; 23(4):380-5.**
**http://www.cancernetwork.com/cme/article/10165/1401764**

Table 1

## Limitations of Traditional Irradiation: Tumor Control and Adverse Effects After 'Conventional' Radiotherapy

| Type of Cancer | Treatment[a] | Tumor Control | Adverse Effects[b] |
|---|---|---|---|
| Glioblastoma[2] | Temozolomide | Median survival 14.6 mo<br>Death in 73.5% by 2 yr | Gr 3/4 nonhematologic toxicity in 31% (most common: fatigue and other constitutional symptoms, rashes and other dermatologic effects, infection, effects on vision, nausea, vomiting) |
| Head and neck: locally advanced, unresectable[3] | Cetuximab | Median survival 49 mo<br>Death in 45% by 3 yr<br>Local failures in 53% by 3 yr<br>Distant metastases in 17% by 3 yr | Gr 3–5 mucosal toxicity in 56%<br>Gr 3–5 dyspahgia in 26%<br>Gr 3–5 dermatitis in 23%<br>Gr 3–5 weight loss in 11% |
| Head and neck: locally advanced, resected[4] | Cisplatin | Median survival 48 mo<br>Local failures in 16%<br>Distant metastases in 20% | Gr 4/5 nonhematologic toxicity in 27% (most common: mucositis, pharyngeal/esophageal toxicity, nausea, vomiting, skin toxicity) |
| Larynx: locally advanced[5] | Cisplatin | Death in 26% by 2 yr<br>Laryngectomy in 12% by 2 yr<br>Distant metastases in 8% by 2 yr | Acute gr 3/4 nonhematologic toxicity in 77% (most common: mucositis, pharyngitis/esophagitis, laryngitis)<br>Dysphagia persisted at 2 yr in 15% |
| Nasopharynx[6] | Chemotherapy | Death in 24% by 3 yr<br>Local failures in 14%<br>Distant metastases in 15% | Gr 3 or worse toxicity in 76% (most common: stomatitis, nausea, vomiting, hearing loss, weight loss) |
| Lung: non–small-cell, locally advanced[7] | Continuous hyperfractionated accelerated radiation therapy | Median survival 16.5 mo<br>Death in 71% by 2 yr<br>Local failures in 77%<br>Distant metastases in 52% | Symptomatic acute pneumonitis in 10%<br>Severe dysphagia persisted at 2 yr in 7% |
| Lung: non–small-cell, locally advanced[8,9] | Chemotherapy before irradiation | Median survival 13.2 mo<br>Death in 68% by 2 yr<br>Local failures in 59%<br>Distant metastases in 39% | Acute gr 3–5 toxicity in 52%<br>Late gr 3–5 toxicity in 3% |
| Lung: small-cell, Limited disease[10] | Chemotherapy | Median survival 23 mo<br>Death in 74% by 5 yr<br>Local failures in 36%<br>Distant metastases in 6% (in addition to local failure) | Acute gr 3–5 esophagitis in 32%<br>Other common nonhematologic toxicity: infection, fever, vomiting, pulmonary effects, weight loss |
| Esophagus[11] | Chemotherapy | Median survival 18 mo<br>Death in 60% by 2 yr<br>Local failures in 55%<br>Distant metastases in 16% | Acute gr 3–5 toxicity in 71% (treatment-related death due to infection in 2%)<br>Late gr 3–5 toxicity in 37% (esophageal stricture, perforation, bleeding) |
| Breast: early, postlumpectomy[12] | Tamoxifen | Death in 7% by 5 yr (2.5% due to breast cancer)<br>Local failures in 3.5% by 8 yr<br>Distant metastases in 4.5% | Gr 3 fatigue in 1%<br>Gr 3 skin erythema in 1% |

Table 1 continued

| Type of Cancer | Treatment[b] | Tumor Control | Adverse Effects |
|---|---|---|---|
| Breast: postmastectomy[13] | Chemotherapy | Death in 53% by 20 yr<br>Local failures in 13% by 20 yr<br>Distant metastases in 52% | Fatal cardiac toxicity in 1% at 20 yr<br>Arm edema in 6%<br>Symptomatic pneumonitis in 0.6% |
| Pancreas: resected[14] | Chemotherapy | Median survival 17 mo<br>Death in 80% by 5 yr<br>Local failures in 23%<br>Regional failures in 7%<br>Distant metastases in 75% | Gr 3 or worse nonhematologic toxicity in 58% (most common: diarrhea, stomatitis, nausea,vomiting) |
| Prostate: early[15] | Brachytherapy | Death in 3% by 5 yr (none due to prostate cancer)<br>Distant metastases in 1% | Acute gr 3 GU bleeding/toxicity in 8%<br>Late gr 3 urinary obstruction/retention in 2%<br>Moderate/severe erectile dysfunction in 9% |
| Prostate: post-prostatectomy[16] | | Median survival 15 yr<br>PSA relapse in 50% by 10 yr<br>Distant metastases in 50% by 15 yr | Toxicity in 24% (most common: urethral stricture in 18%, urinary incontinence in 6.5%, rectal complications in 3%) |
| Prostate: locally advanced, intermediate risk[17,18] | Androgen deprivation | Death in 12% by 5 yr (none due to prostate cancer) | Gr 3 erectile dysfunction in 26%<br>Gr 3 urinary bleeding/incontinence in 4%<br>Gr 3 diarrhea/rectal bleeding in 4% |
| Prostate: locally advanced, high risk[19-21] | Androgen deprivation | Death in 22% by 5 yr (6% due to prostate cancer)<br>Local failures in 2%<br>Distant metastases in 10% | Fatal urinary stricture in 1%<br>Gr 3 toxicity in 2.7% (urinary stricture/toxicity or small bowel obstruction)<br>Gr 2 toxicity in 19% (most common: cystitis, hematuria, incontinence, proctitis, leg edema)<br>Erectile dysfunction in 68% |
| Cervix[22] | Chemotherapy | Death in 27% by 5 yr<br>Local failures in 19%<br>Distant metastases in 14% | Acute gr 3–5 nonhematologic toxicity in 11% (most common: nausea, vomiting, diarrhea)<br>Late gr 3/4 toxicity in 12% (most common: bowel and urinary effects) |
| Endometrium: post-hysterectomy[23] | | Death in 19% by 5 yr (9% due to endometrial cancer)<br>Local failures in 4% | Gr 3/4 toxicity in 2% (most common: bowel obstruction)<br>Gr 2 toxicity in 6%<br>Gr 1 toxicity in 16% |
| Rectum: locally advanced[24] | Chemotherapy | Death in 24% by 5 yr<br>Local failures in 6%<br>Distant metastases in 36%<br>Abdominoperineal resection necessary in 17% | Acute gr 3/4 nonhematologic toxicity in 27% (most common: diarrhea, skin toxicity)<br>Long-term gr 3/4 toxicity in 14% (most common: diarrhea, bowel obstruction, anastamotic stricture, bladder problems) |
| Anal canal[25] | Chemotherapy | Death in 25% by 5 yr<br>Colostomy necessary in 10% by 5 yr<br>Distant metastases in 15% | Acute gr 3 or worse nonhematologic toxicity in 74% (most common: skin, GI toxicity)<br>Long-term gr 3 or worse toxicity in 11% (most common: effects on the bowels, skin, subcutaneous tissues) |

[a]Besides "conventional" irradiation. [b]The scoring systems used varied among the various papers.

Gr = grade; GI = gastrointestinal; GU = genitourinary; PSA = prostate-specific antigen.

1. Andrews DW, Scott CB, Sperduto PW, et al: Whole brain radiation therapy with or without stereotactic radiosurgery boost for patients with one to three brain metastases: Phase III results of the RTOG 9508 randomised trial. Lancet 363:1665-1672, 2004.
2. Stupp R, Mason WP, van den Bent MJ, et al, European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987-996, 2005.
3. Bonner JA, Harari PM, Giralt J, et al: Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. N Engl J Med 354:567-578, 2006.
4. Cooper JS, Pajak TF, Forastiere AA, et al, Radiation Therapy Oncology Group 9501/Intergroup: Postoperative concurrent radiotherapy and chemotherapy for high-risk squamous-cell carcinoma of the head and neck. N Engl J Med 350:1937-1944, 2004.
5. Forastiere AA, Goepfert H, Maor M, et al: Concurrent chemotherapy and radiotherapy for organ preservation in advanced laryngeal cancer. N Engl J Med 349:2091-2098, 2003.
6. Al-Sarraf M, LeBlanc M, Giri PG, et al: Chemoradiotherapy versus radiotherapy in patients with advanced nasopharyngeal cancer: Phase III randomized Intergroup study 0099. J Clin Oncol 16:1310-1317, 1998.
7. Saunders M, Dische S, Barrett A, et al: Continuous, hyperfractionated, accelerated radiotherapy (CHART) versus conventional radiotherapy in non-small cell lung cancer: Mature data from the randomised multicentre trial. CHART Steering committee. Radiother Oncol 52:137-148, 1999.
8. Sause W, Kolesar P, Taylor S IV, et al: Final results of phase III trial in regionally advanced unresectable non-small cell lung cancer: Radiation Therapy Oncology Group, Eastern Cooperative Oncology Group, and Southwest Oncology Group. Chest 117:358-364, 2000.
9. Komaki R, Scott CB, Sause WT, et al: Induction cisplatin/vinblastine and irradiation vs. irradiation in unresectable squamous cell lung cancer: Failure patterns by cell type in RTOG 88-08/ECOG 4588. Radiation Therapy Oncology Group. Eastern Cooperative Oncology Group. Int J Radiat Oncol Biol Phys 39:537-544, 1997.
10. Turrisi AT 3rd, Kim K, Blum R, et al: Twice-daily compared with once-daily thoracic radiotherapy in limited small-cell lung cancer treated concurrently with cisplatin and etoposide. N Engl J Med 340:265-271, 1999.
11. Minsky BD, Pajak TF, Ginsberg RJ, et al: INT 0123 (Radiation Therapy Oncology Group 94-05) phase III trial of combined-modality therapy for esophageal cancer: High-dose versus standard-dose radiation therapy. J Clin Oncol 20:1167-1174, 2002.
12. Fyles AW, McCready DR, Manchul LA, et al: Tamoxifen with or without breast irradiation in women 50 years of age or older with early breast cancer. N Engl J Med 351:963-970, 2004.
13. Ragaz J, Olivotto IA, Spinelli JJ, et al: Locoregional radiation therapy in patients with high-risk breast cancer receiving adjuvant chemotherapy: 20-year results of the British Columbia randomized trial. J Natl Cancer Inst 97:116-1126, 2005.
14. Regine WF, Winter KA, Abrams RA, et al: Fluorouracil vs gemcitabine chemotherapy before and after fluorouracil-based chemoradiation following resection of pancreatic adenocarcinoma: A randomized controlled trial. JAMA 299:1019-1026, 2008.
15. Lawton CA, DeSilvio M, Lee WR, et al: Results of a phase II trial of transrectal ultrasound-guided permanent radioactive implantation of the prostate for definitive management of localized adenocarcinoma of the prostate (Radiation Therapy Oncology Group 98-05). Int J Radiat Oncol Biol Phys 67:39-47, 2007.
16. Thompson IM Jr, Tangen CM, Paradelo J, et al: Adjuvant radiotherapy for pathologically advanced prostate cancer: A randomized clinical trial. JAMA 296:2329-2335, 2006.

17. DAmico AV, Manola J, Loffredo M, et al: 6-month androgen suppression plus radiation therapy vs radiation therapy alone for patients with clinically localized prostate cancer: A randomized controlled trial. JAMA 292:821-827, 2004.
18. DAmico AV, Chen MH, Renshaw AA, et al: Androgen suppression and radiation vs radiation alone for prostate cancer: A randomized trial. JAMA 299:289-295, 2008.
19. Bolla M, Gonzalez D, Warde P, et al: Improved survival in patients with locally advanced prostate cancer treated with radiotherapy and goserelin. N Engl J Med 337:295-300, 1997.
20. Bolla M, Collette L, Blank L, et al: Long-term results with immediate androgen suppression and external irradiation in patients with locally advanced prostate cancer (an EORTC study): A phase III randomised trial. Lancet 360:103-108, 2002.
21. Ataman F, Zurlo A, Artignan X, et al: Late toxicity following conventional radiotherapy for prostate cancer: Analysis of the EORTC trial 22863. Eur J Cancer 40:1674-1681, 2004.
22. Morris M, Eifel PJ, Lu J, et al: Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer. N Engl J Med 340:1137-1143, 1999.
23. Creutzberg CL, van Putten WL, Koper PC, et al: Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: Multicentre randomised trial. PORTEC Study Group. Post Operative Radiation Therapy in Endometrial Carcinoma. Lancet 355:1404-1411, 2000.
24. Sauer R, Becker H, Hohenberger W, et al; German Rectal Cancer Study Group: Preoperative versus postoperative chemoradiotherapy for rectal cancer. N Engl J Med 351:1731-1740, 2004.
25. Ajani JA, Winter KA, Gunderson LL, et al: Fluorouracil, mitomycin, and radiotherapy vs fluorouracil, cisplatin, and radiotherapy for carcinoma of the anal canal: A randomized controlled trial. JAMA 299:1914-1921, 2008.
26. Souhami L, Seiferheld W, Brachman D, et al: Randomized comparison of stereotactic radiosurgery followed by conventional radiotherapy with carmustine to conventional radiotherapy with carmustine for patients with glioblastoma multiforme: Report of Radiation Therapy Oncology Group 93-05 protocol. Int J Radiat Oncol Biol Phys 60:853-860, 2004.
27. Pow EH, Kwong DL, McMillan AS, et al: Xerostomia and quality of life after intensity-modulated radiotherapy vs. conventional radiotherapy for early-stage nasopharyngeal carcinoma: Initial report on a randomized controlled clinical trial. Int J Radiat Oncol Biol Phys 66:981-991, 2006.
28. Kam MK, Leung SF, Zee B, et al: Prospective randomized study of intensity-modulated radiotherapy on salivary gland function in early-stage nasopharyngeal carcinoma patients. J Clin Oncol 25:4873-4879, 2007.
29. Donovan E, Bleakley N, Denholm E, et al; Breast Technology Group: Randomised trial of standard 2D radiotherapy (RT) versus intensity modulated radiotherapy (IMRT) in patients prescribed breast radiotherapy. Radiother Oncol 82:254-64, 2007.
30. Pignol JP, Olivotto I, Rakovitch E, et al: A multicenter randomized trial of breast intensity-modulated radiation therapy to reduce acute radiation dermatitis. J Clin Oncol 26:2085-2092, 2008.
31. Shipley WU, Verhey LJ, Munzenrider JE, et al: Advanced prostate cancer: The results of a randomized comparative trial of high dose irradiation boosting with conformal protons compared with conventional dose irradiation using photons alone. Int J Radiat Oncol Biol Phys 32:3-12, 1995.
32. Gardner BG, Zietman AL, Shipley WU, et al: Late normal tissue sequelae in the second decade after high dose radiation therapy with combined photons and conformal protons for locally advanced prostate cancer. J Urol 167:123-126, 2002.

## WHAT ARE THE MOST SERIOUS ADVERSE EVENTS DURING AND AFTER RADIATION THERAPY?

*Bhadrasain Vikram, MD*

## GLIOBLASTOMA

*Stupp, NEJM 352:987-96, 2005.*

- ≥Grade 3 non-heme toxicity in 31%. Most common:
  - *Fatigue & other constitutional symptoms*
  - *Rashes & other skin effects*
  - *Infection*
  - *Effects on vision*
  - *Nausea & Vomiting*

## HEAD & NECK

*Bonner, NEJM 354:567-78, 2006.*

- ≥Grade 3 toxicity:
  - *Mucosal 56%*
  - *Dysphagia 26%*
  - *Dermatitis 23%*
  - *Weight loss 11%*

## SMALL CELL LUNG - LD

*Turrisi, NEJM 340:265-71, 1999.*

- ≥Grade 3 non-heme toxicity:
  - *Esophagitis in 32%*
  - *Infection*
  - *Fever*
  - *Vomiting*
  - *Pulmonary effects*
  - *Weight loss*

## ESOPHAGUS

*Minsky, JCO 20:1167-74, 2002.*

- ≥Grade 3 acute toxicity in 71%.
- ≥Grade 3 late toxicity in 37%.
  - *Esophageal strictures, perforations, bleeding.*

## BREAST: POST-LUMPECTOMY

*Fyles, NEJM 351:963-970, 2004.*

- ≥Grade 3 toxicity:
  - *Fatigue 1%*
  - *Skin erythema 1%*

## BREAST: POST-MASTECTOMY

*Ragaz, JNCI 97:116-1126, 2005.*

- ≥Grade 3 late toxicity @ 20 years:
  - *Cardiac 1%*
  - *Arm edema 6%*
  - *Symptomatic pneumonitis 0.6%*

## PANCREAS: POST-OP

*Regine, JAMA 299:1019-1026, 2008.*

- ≥Grade 3 non-heme toxicity in 58%. Most common:
  - *Diarrhea*
  - *Stomatitis*
  - *Nausea & Vomiting*

## PROSTATE: LOW-RISK

*Lawton, IJROBP 67:39-47, 2007.*

- ≥Grade 3 acute toxicity:
  - *GU bleeding/toxicity 8%*
- ≥Grade 3 late toxicity:
  - *Erectile dysfunction 9%*
  - *GU obstruction/retention 2%*

## PROSTATE: HIGH-RISK

*Bolla, Lancet 360:103-108, 2002.*

- ≥Grade 3 toxicity:
  - *Erectile dysfunction 68%*
  - *GI/GU toxicity 3.7%*

## CERVIX

*Morris, NEJM 340:1137-1143, 1999.*

- ≥Grade 3 acute non-heme toxicity in 11%. Most common:
  - *Nausea & vomiting*
  - *Diarrhea*
- ≥Grade 3 late toxicity in 12%. Most common:
  - *Bowel & Urinary toxicity*

## RECTUM

*Sauer, NEJM 351:1731-1740, 2004.*

- ≥Grade 3 non-heme acute toxicity in 27%. Most common:
  - *Diarrhea*
  - *Dermatitis*
- ≥Grade 3 late toxicity in 14%. Most common:
  - *Diarrhea*
  - *Bowel obstruction/strictures*
  - *Bladder problems*

## ANAL

*Ajani, JAMA 299:1914-1921, 2008.*

- ≥Grade 3 non-heme acute toxicity in 74%. Most common:
  - *Skin toxicity*
  - *Bowel toxicity*
- ≥Grade 3 late toxicity in 11%. Most common:
  - *Bowel toxicity*
  - *Skin & Subcutaneous*

## SUMMARY

- Serious adverse events frequently occur during and after radiation therapy in many common cancers.
- The impact of advanced technologies (IMRT, Protons, etc.) in that regard has until now been quite modest.

**Andrew M. Trotti, M.D.**

(No summary received)

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ART-RIM Workshop
1-25-10
Bethesda, MD

# Toxicities in H and N Cancer: A Shifting Landscape

Andy Trotti III, MD
H. Lee Moffitt Cancer Center
& Research Institute

Andy Trotti, MD
## Background and Interests

- Radiation Oncologist, H. Lee Moffitt Cancer Center
- H&N Cancer Clinical Trials
- Adverse Events: Assessment, Reporting and Interventions
- Member NCCN H&N Guidelines Committee
- Co-Chair of NCI and RTOG H&N Committees

## H&N: Rapidly Shifting Landscape

- Epidemiology: Rise of HPV; decline of smoking
- Increasing use of complex/aggressive chemoradiation programs
- Introduction of biologics
- Rapid evolution of radiation technology
- Increasing toxicity and supportive care issues
- Declining Toxicities from IMRT

Head and Neck
## *Traditionally: Most Common and Serious Toxicities*

- Xerostomia and dental complications
- Mucositis
- Swallowing Disorders

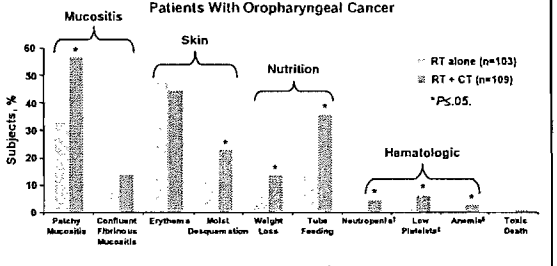
1st Combined H&N Symposium ASTRO/ASCO/AHNS
January 19, 2007, Palm Springs, CA

## Increasing Toxicity in Non-operative Head and Neck Treatment: *Investigations and Interventions*

Soren Bentzen, Ernest A. Weymuller, David Rosenthal, Andy Trotti, MD

Published IJORBP September 2007

## Increased Acute Toxicity with ChemoRT

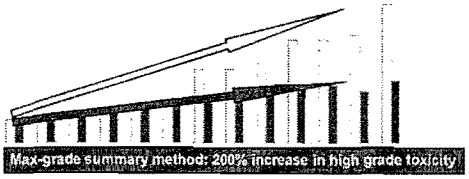



†Neutrophil count <0.9 cells/mm3; ‡platelet count <50 cells/mm3; §hemoglobin level <8 g/100 mL
RT alone = cobalt-60 (total dose, 70 Gy); RT + CT = RT + 5-FU and carboplatin.

Calais G, et al. *J Natl Cancer Inst.* 1999;91:2081-2086.

## Toxicity Burden Measures: Relative T-Value vs Relative Max Grade Value

T-Score (ALL events): >500% increase in high grade toxicity



Max-grade summary method: 200% increase in high grade toxicity

Trotti & RTOG; Lancet Oncol 2007

## IMRT trials: reduced xerostomia

| | | | | |
|---|---|---|---|---|
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## Amifostine Registration Trial

Randomization* (N = 303)

- RT + amifostine IV 200 mg/m²
- RT

FDA Approved for Reduction of Xerostomia in Postoperative RT

RT alone (33%); Postoperative RT (67%)

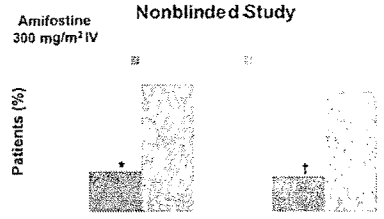
*Nonblinded Study

RT = radiotherapy.
Brizel DM et al. *J Clin Oncol*. 2000;18:3339-3345.

9

## Phase 2 RCT ± Amifostine

Nonblinded Study




*Grade 2 at 3 months $P = .0001$
†Grade 3 at 4 weeks $P = .0003$
RCT = radiochemotherapy.
Adapted from Antonadou D et al. Int *J Radiat Oncol Biol Phys*. 2002;52:739-747.

10

## Advances in Radiation Therapy Technology

## IMRT in H&N Cancer

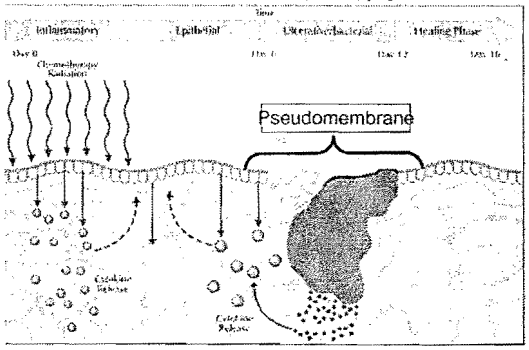
- IMRT now widely adopted and utilized
- >90% of patients on H&N trials get IMRT
- Requires advanced peer review and QA measures
- Permits wider variations in dose plan and delivery
- Next phase: integration of IGRT (daily imaging)

## Mucositis and associated changes

### *Acute consequences of mucositis*

- *Pain*
- *Impaired oral intake*
- *Swallowing Disorders*
- *Increased secretions*
- *Gagging, nausea and vomiting*
- *Taste alteration*

### Phases of Mucositis




Adapted from Sonis

### Radiation-Induced Mucositis

- Pain and Ulceration
- Ulcerative mucositis may occur anywhere in the irradiated mucosal volume

## *Late* Mucosal Reactions

### *Late and consequential effects of mucositis*

- *Oral compromise (eating/speech)*
- *Chronic Swallowing Dysfunction*
- *Impaired Taste*
- *Mucosal sensitivity*
- *Chronic weight loss*
- *Soft tissue and bone necrosis*

# Radiation Dermatitis

## Head and Neck
## *Late Effects*

- Xerostomia
- Trismus
- Swallowing disorders
- Fibrosis
- Hypothyroidism
- Spinal cord

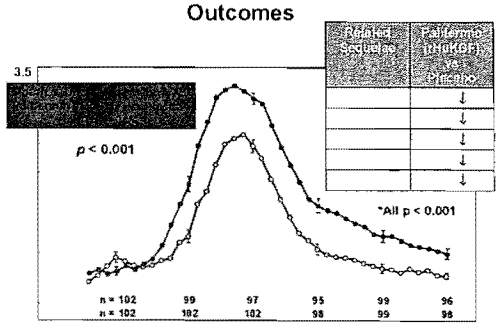
# KGF

## Recombinant Human Keratinocyte Growth Factor (Palifermin)

- Fibroblast growth factor family (FGF-7)
- ↑ Epithelial cell proliferation and differentiation
  - Mucosa, type II pneumocytes, salivary
- Upregulates protective mechanisms
  - GSH reductase
- Preclinical: Decreases mucositis from RT & CT*

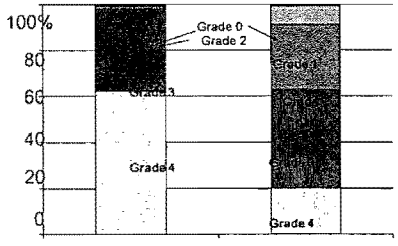
* Dorr, *IJROBP*, 46(729), 2000

## KGF in TBI/BMT: Patient-Reported Outcomes



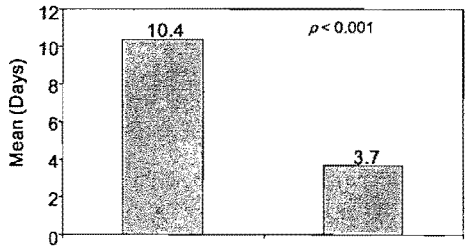

Spielberger, *NEJM* 351:2590-2598, 2004

## KGF in TBI/BMT Mucositis Incidence




Spielberger, *NEJM* 351:2590-2598, 2004

## Duration of Severe Oral Mucositis (Grade 3-4 WHO)




*Spielberger, NEJM 351:2590-2598, 2004*

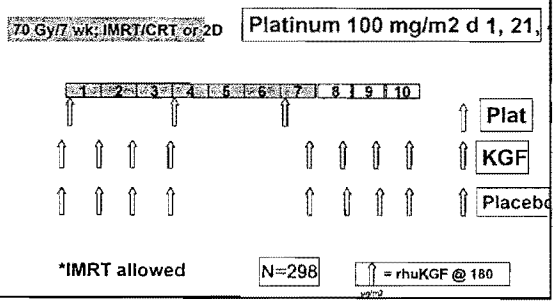
## KGF Scheduling Designs in H&N


| Pre | Early | Post |
|---|---|---|
| 1Dose | 3 doses | 3-4 doses |

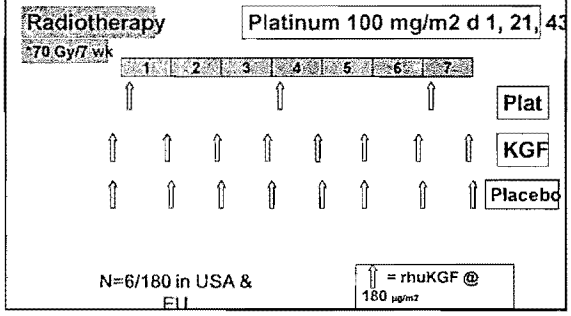
Continuous

**8 doses**

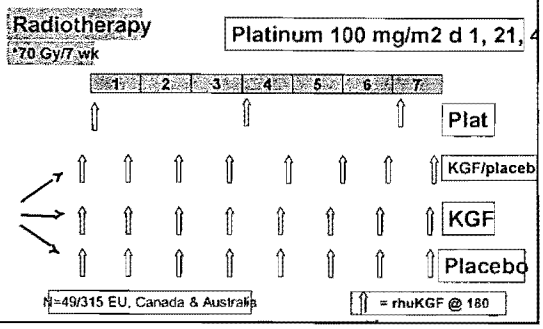
## RTOG 0435 Phase III Study "concurrent-early & post treatment"




## Amgen Phase III "Unresected"Study (0402) ("concurrent continuous")




## Amgen Phase III "post-op" Study (0118) "concurrent-early vs continuous"

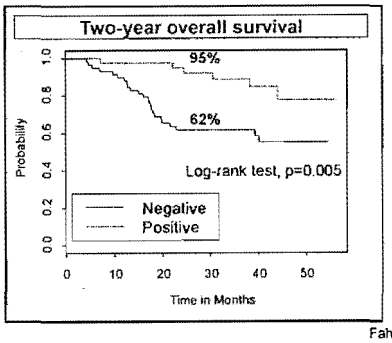



## Shifting Epidemiology

## Two distinct head and neck cancers

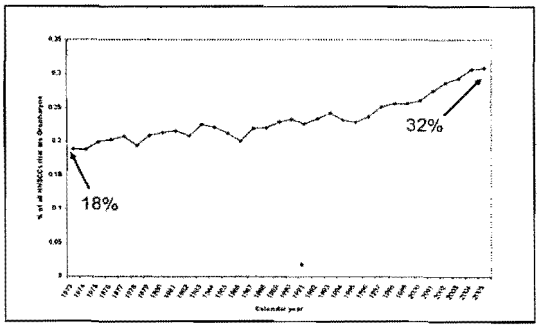
Adapted from M. Gillison

## Tumor HPV status and survival




Fakhri 2008 JCO

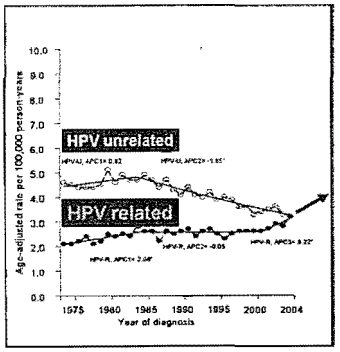
## Proportion of all HNSCC that are oropharynx, U.S. 1973-2005




Chaturvedi 2008 JCO

## Incidence trends

- Inc idence increasing for HPV-related
- Incidence decreasing for HPV-unrelated
- Equalization in 2004




Chaturvedi 2008 JCO

## NCI CTPM Consensus

*Principles of HPV Trial Development*

- HPV+ is sufficiently different disease that it requires separate trials (c.f. HPV neg)
- Treatment de-escalation questions are appropriate
- Insufficient number, low number of events, of HPV+ patients for a phase III trial
- Stratify for smoking
- Need central reference lab with quick turn around (BISQFP funding; "Integral")
- All patients should be treated with IMRT technology as SOC

## Conclusions

- Shifting landscape: epidemiology and therapy
- Increasing number and variety of treatment options
- Increasing toxicity requires more supportive care and longer recovery time
- Complexity of new technology is associated with variations in targeting, delivery and new patterns of toxicity
- Chronic dysphagia may be declining due to IMRT

Mechanisms of RT toxicity

The response to radiation is a temporal one with sequential molecular events proceeding up to and even beyond the point of the development of overt injury. However, the relative significance of individual events in determining the final outcome, either a normal response or a pathologic one, remains unclear. Essentially, radiation creates a wound that initiates a healing response. In the majority of cases, the injury produced by RT exposure resolves with no significant clinical manifestations. In other cases, however, overt injury develops. Evidence suggests that radiation-induced injury may be the result of an abnormal wound healing response. Wound healing is often divided into 3 phases: injury, inflammation and repair. The injury following radiation may occur in response to the immediate generation of reactive oxygen and nitrogen species, with resultant damage to DNA, lipids and proteins, resulting in the death of epithelial and endothelial cells. This initial response may also be characterized by a transient decrease in organ perfusion. In response to this injury, inflammatory cells are recruited, which release and activate a number of cytokines, chemokines and growth factors, leading to further cellular recruitment, and activation of signaling pathways involved in tissue repair. In normal wound healing, the inflammatory response eventually subsides after repair is complete. However, in an abnormal wound healing response, such as that following radiation, the inflammatory response is dysregulated either in duration or in degree, leading to a cascade of signaling events, chronic inflammation, tissue hypoxia and fibrosis with loss of parenchymal cells, eventually leading to the atrophy and fibrosis characteristic of late radiation injury.

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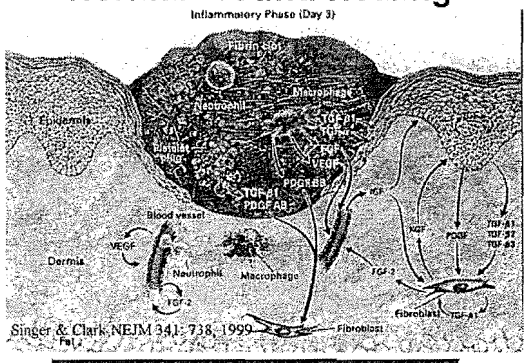
MCV

# Mechanisms of RT Toxicity

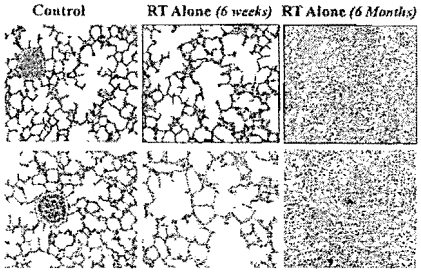
*Mitchell S. Anscher, MD*
*Department of Radiation Oncology*
*Virginia Commonwealth University*
*Medical College of Virginia Hospitals*
*Richmond, Virginia USA*

# Normal Wound Healing



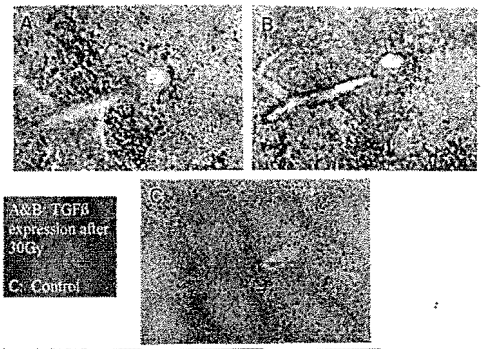

VCU

# Radiation-Induced Lung Injury




VCU

# Lack of Fibrosis in Animal Models of RT Induced Liver Injury

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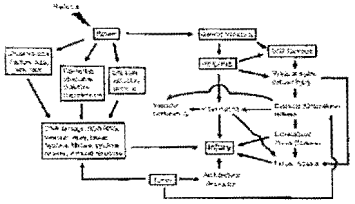
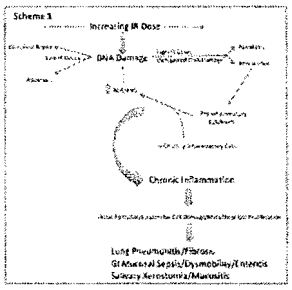
# Factors Contributing to RT-induced Injury



Graves et al Sem in Rad Onc in press

VCU

# Role of Inflammation

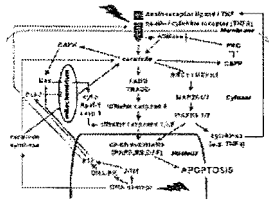



**Key elements**
- DNA damage (initiation)
- Inflammatory cell infiltration (amplification)
- ROS/RNS generation (transmission)

Courtesy R. Mikkelsen 2009

VCU

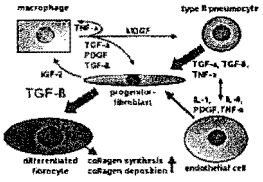
## DNA Damage-independent and – Dependent Pathways of Endothelial Cell Apoptosis




Rodemann & Blaese Sem Rad Onc 17: 81, 2007

VCU

## Multicellular Interactions in Connective Tissue Remodelling




Rodemann & Blaese Sem Rad Onc 17: 81, 2007

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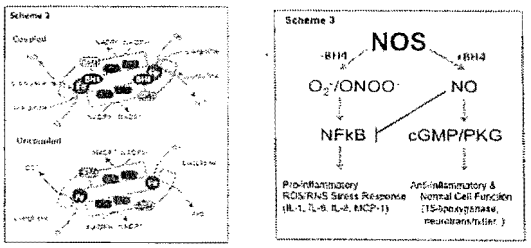
## Impact of NOS Activity




Courtesy R. Mikkelsen 2009

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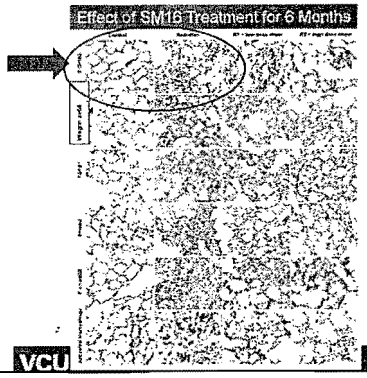
## Evidence for Sustained Oxidative Stress




Anscher et al IJROBP 71:358, 2008

VCU

## Hypoxia in RT-Induced Lung Injury

QuickTime™ and a decompressor are needed to see this picture.

VCU

## Time Course of Protein Expression and Tyr Nitration

QuickTime™ and a decompressor are needed to see this picture.

Yakovlev et al submitted 2009

VCU

## Chronology of RT Induced Injury

QuickTime™ and a decompressor are needed to see this picture

Graves et al. Sem in Rad Onc, in press

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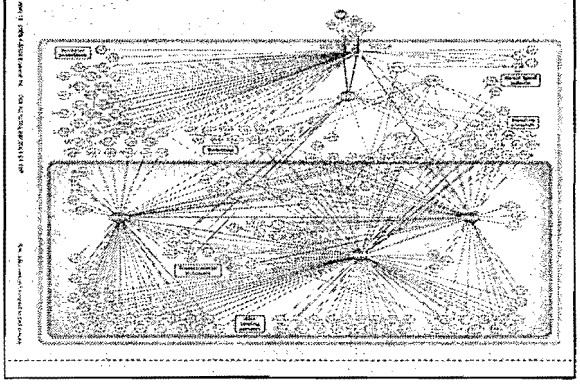
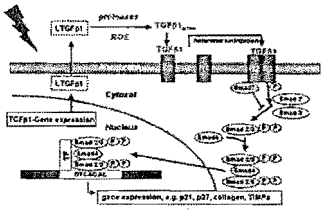
## SnapShot: The TGFβ Pathway Interactome



## Tgf-ß1-dependent Signal Transduction and Gene Expression



Rodemann & Blaese Sem Rad Onc 17: 81, 2007

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## Smad Kos Resistant To RT

QuickTime™ and a decompressor are needed to see this picture.

Table 1. Qualitative Analysis of Histological Appearance of Irradiated Lor Skin Samples (50 Gy) from WT, Het and KO Mice

| | Number of animals (%) | | |
|---|---|---|---|
| | WT | Het | KO |
| Epidermis | | | |
| Ulceration | 10/10 (100) | 10/10 (100) | 6/7 (86) |
| Severe hyperplasia | 9/10 (90) | 8/10 (80) | 5/7 (71) |
| Moderate hyperplasia | 1/10 (10) | 2/10 (20) | 2/7 (29) |
| Normal | 0/10 (0) | 0/10 (0) | 0/7 (0) |
| Dermis | | | |
| Severe | 10/10 (100) | 10/10 (100) | 6/7 (86) |
| Moderate | 0/10 (0) | 0/10 (0) | 1/7 (14) |
| Mild | 0/10 (0) | 0/10 (0) | 0/7 (0) |

*Ulceration located on sides of mice where restraint occurred.

Flanders et al. Am J Pathol 160: 1057, 2002

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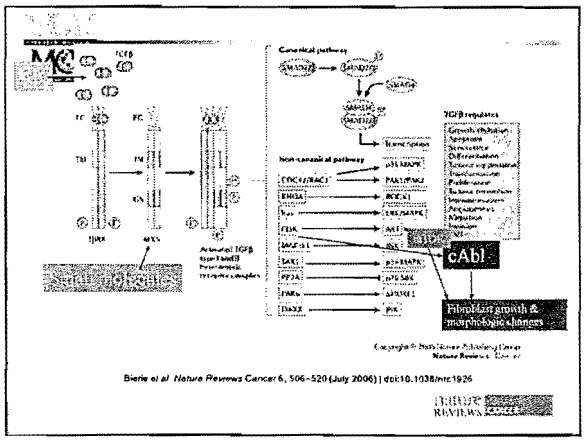
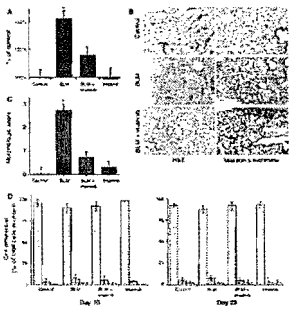



Biernacka et al. Nature Reviews Cancer 6, 506–520 (July 2006) | doi:10.1038/nrc1926

## Imatinib Reduces Hydroxyproline Content and Prevents Histopathologic Changes in BLM-treated Lungs



Daniels, C.E. et al. J. Clin. Invest. 2004;114:1308-1316

JCI

VCU

## Model of RT Injury



Fleckenstein et al Sem Rad Onc 17: 89, 2007

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## Conclusions

- RT injury is a dysregulation of wound healing
- Multiple cellular compartments involved
- Multiple pathways involved; TGFß key mediator of fibrosis
- Multiple potential targets for intervention

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## ROS/RNS Chemistry



http://circ.ahajournals.org/content/vol108/issue16/images/large/4FF1.jpeg

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A Patient's Perspective
David H. Klein, Patient Advocate

Meeting on Advanced Radiation Therapeutics and
Radiation Injury Mitigation
Rockville, Maryland
January 25, 2010

Radiation Research Program
Division of Cancer Treatment & Diagnosis
National Cancer Institute
National Institutes of Health

Thank you, Drs. Okunieff, Vikram and Movsas. It is a privilege to be here today sharing my thoughts with this distinguished panel of radiation oncologists.

I have worked as health care administrator and health insurance executive since the late 1960's. For the last six years, I have served as the CEO of Excellus BlueCross BlueShield, a not for profit health plan headquartered in Rochester, NY that serves nearly two million people.

I view my job as a health insurer to provide access to the medical care that is delivered by you and your colleagues.

It has been forty plus years since entering this industry, yet I still stand in awe of what you collectively do to enhance the quality of life and to save lives. Thus for me, you're providing a few minutes to share my thoughts is indeed an honor.

However, I am not here today as a health care administrator or health insurance executive. Rather, I am here as a patient advocate. I am here because my wife has advanced head and neck cancer. I am here with her very strong encouragement to share her story.

My understanding of the purpose of today's workshop is to aid NCI in setting research funding priorities in radiation oncology. Said simply,

in a world of scarce resources, how much focus should there be on just killing malignant cells and increasing survival and extending life versus perhaps accepting a lower survival rate and providing patients with a higher quality of life for however long they will live?

Should more be done to mitigate the usual injury caused by radiotherapy to normal tissues?

I have been invited here today to offer the point of view of a patient and her family who has faced and frankly continues to face this very difficult choice of quality of life versus length of life.

Allow me to start with my observations and conclusions. I'll follow with my and my wife's experiences to offer support for suggestions being made.

1. To start, I belabor the obvious. Every patient is different and therefore their preferences will be different.

2. There is a spectrum of patient desire driven by values, age, general health and other factors. The range goes from survival regardless of impact on the quality of life to a patient receiving palliative but no curative care from the onset of the cancer diagnosis.

3. Patient preferences change over time. There is usually more interest in fighting for survival early on. However, as cancer progresses and quality of life deteriorates, there is often more acceptance of the need to balance the fight with maximizing the quality of life – for however long that is.

4. However, most physicians and nurses are more comfortable dealing with survival as opposed to quality of life issues. There is often a "never say die" attitude. More time is spent describing treatment and its likelihood of success than educating patients regarding the probability of side effects, how they'll affect a patient's life and to what extent they'll be mitigated. As a result, patients' hopes and preferences may be in conflict with those of their clinical caregivers, and, as we all know, patients will usually accede to providers' preferences.

5. Physicians and nurses that do try to educate patients regarding benefits and risks of treatment are more comfortable in dealing with physically observable side effects like functional impairment than they are with emotional and social issues like loneliness, isolation, concern about changes in personal appearance as a result of radiation therapy, and fear of pain or of the process of dying.

6. Many physicians and nurses believe that educating patients or even their advocates who may have medical grounding is challenging and overly time consuming. This leads the clinicians to explicitly or implicitly make judgments on behalf of their patients regarding preference for quality of life versus length of life.

7. Patient advocates can and do get it wrong. Caring, intelligent, sensitive, and selfless as they may be, they can misread patient preferences or infuse their own values. They may have a hard time letting go. The ultimate decision regarding treatment must be reserved for the patient. It is his or her body and soul.

8. We need a different definition of success or victory -- and it is not in every case beating cancer and surviving. My suspicion is that societies in other parts of the world may do a better job than we do in dealing with this "death with dignity" question.

So where does all of this lead me, as they pertain to NCI radiation oncology research funding priorities?

1. Creative, new technologies like IMRT that spare more normal tissue are great. Don't stop developing them. Candidly though, having said that my hope is at some point the hematologist-oncologists will achieve a breakthrough and render surgical or radiation oncology unnecessary through their work in targeted therapies. However, I do believe we are a long way off from that day, so don't stop research and development.

2. On a parallel track, you should work even harder on injury mitigation. Radiotherapy, especially in parts of the body where

there is extensive neurological or other function like the head and neck, can be ravaging. The damage is not only physical and functional but also emotional. These devastating side effects can and do have a catastrophic impact on the quality of life. Just the possibility they may occur can also drive a patient away from curative treatment.

3. You should enhance efforts to educate patients and their advocates regarding the risks and benefits of treatment so they will fully comprehend their options. Achieving a real level of informed consent will aid in therapeutic compliance.

However, in some if not many cases, you should anticipate that higher quality of life will win out over just surviving for a while longer.

4. Physicians are not trained to be cancer care educators or, generally, how to discuss treatment options that may not extend life. There is a workforce development need for specialized, properly trained allied health professionals, as well as physicians, who would no doubt be assisted with special teaching aids that facilitate patient understanding of risks and benefits.

Palliative care with its capacity to not only educate but to also provide emotional and psychosocial support could play a significant role here. One care delivery model worthy of consideration is to strongly encourage the patient and their family or friends to have very early on in cancer treatment a palliative care consult. Physicians, nurses, social workers and other allied health professionals who specialize in palliative care confront everyday quality versus length of life questions.

I drew these observations and conclusions by serving as the patient advocate for my wife, Linde. I'll begin with her relevant medical history.

Linde has been battling advanced head and neck cancer since mid-2008. Here is a recent picture of Linde. Our home is in Rochester, NY, which explains how Dr. Okunieff and I found each other. Linde is

a 56 year old Caucasian with no history of significant prior illness. She has always taken good care of herself – she eats well, exercises, doesn't smoke, drinks only socially and maintains an emotionally healthy life. She has always taken great pride in her appearance. She is the very loving step-mom to my two adult children and the doting step-grandmother to our two grandchildren.

Linde first noticed what turned out to be symptoms of oral cancer in late 2007. There was soreness in her mouth and a small lump below her ear.

A Rochester community ENT on July 17, 2008, diagnosed Linde as having either Stage III or Stage IV(a) squamous cell carcinoma with the primary site being the right retrogone trimolar region.

I will never forget the gut-wrenching power of those words. I always feel a pain in my stomach when I recall the day they were spoken.

The pathology report provided after surgery confirmed Stage IV(a) with a classification of T4aN2bM0.

The community ENT prescribed a segmental mandibulectomy with reconstruction using a fibula free flap graft. This would be followed by chemoradiotherapy. The ENT noted a 50-60% five year survival rate but also offered the standard qualifications about the data not being risk-adjusted in any way and that each patient is different.

We were heartened by the 50-60% survival statistic knowing that Linde was otherwise in excellent health. Linde was committed to beating this beast. She was prepared to mount the hardest, most valiant fight a patient could.

Given the low local volume for this kind of procedure, we looked to other potential sites for initial treatment. We visited Wilmot, Dana Farber and Roswell. We had records reviewed at MD Anderson. We spoke with Memorial Sloan Kettering and Beth Israel in New York City.

The findings of this extensive research were generally consistent across the cancer centers. They all recommended segmental mandibulectomy followed by chemoradiotherapy.

With a treatment plan chosen, we narrowed the selection to Dana Farber and Roswell because both were relatively close by, used multidisciplinary approaches and had high volumes of cases. We finally chose Roswell because it was closer to home and they agreed to cooperate with Wilmot regarding post-surgical chemoradiotherapy.

Roswell also was the only site that meticulously walked us through the risks and benefits of alternative treatment plans. This took almost two hours!

Linde also "connected" very well with her surgeon at Roswell, creating the sense of trust with her physician that is so critical in successful cancer care.

The Roswell surgeon described the dissection and reconstruction. He noted that her jaw would be wired shut due to the graft and a PEG tube would be placed. He noted the procedure typically takes 11 to 13 hours.

The surgeon explained that Linde would permanently lose sensation on the right half of her face including her ear due to nerve dissection. She might also permanently lose the ability to turn her head depending on the extent of the neck dissection needed to secure clear margins. Her left leg, the fibula donor site, would also be smaller due to the bone being removed. Facial swelling would be significant but after a year or so, symmetry would return – there would be no deformity.

None of this fazed Linde. She was single minded in her pursuit of survival.

Despite his seeming thoroughness, the Roswell surgeon did not reference to the possibility of trismus nor dysphagia...not that their mention would have then changed anything.

The Roswell experience was terrific. Her surgery was August 14, 2008 and she was discharged on August 27. The team there could not have performed better. She left thinking we had beaten the cancer. Again, survival at any cost!

Linde was seen on September 3, 2008 at Wilmot for her radiation oncology intake. There was again a good explanation of some but not all of the side effects of the planned chemoradiotherapy. The treatment plan was to include 32 sessions of IMRT using a Tomotherapy machine. Cisplatin was to be used for concurrent chemotherapy.

Possible radiation therapy side effects that were emphasized and indeed suffered by Linde included in the short term: pain, fatigue, radiation dermatitis, mucositis, thrush, weight loss and loss of taste; and in the long term, xerostomia due to loss of her right side salivary gland. Linde was told had she still not had her PEG tube, one would have been placed to aid her in maintaining appropriate nutrition through the seven weeks of treatment.

An additional side effect Linde was told to anticipate due to the chemotherapy was nausea.

Nothing was said about radiotherapy causing possible impaired hearing, trismus, dysphagia, hypothyroidism or additional xerostomia caused by loss of her left side salivary gland. Linde suffers all of these problems today.

Linde was offered but declined use of the monoclonal antibody, cetuximab. She opted against using it due to the possible acne-like skin rash side effect.

As you can observe from Linde's decision regarding the cetuximab, once informed, she did begin to make quality of life versus survival decisions. She did not at that point automatically opt for the most aggressive treatment. There was the beginning of a change in attitude in just the few weeks post surgery as side effects increased.

Linde's recovery from the surgery and chemoradiotherapy has been challenging. However, despite all of her very obvious suffering, she maintains a very positive, can-do attitude and rarely complains. While in Rochester, Dr. Okunieff was one of her physicians and will surely attest to Linde's positivity.

Linde continues to present this very upbeat spirit despite still not being able to swallow, which is clearly the most torturous effect of all of her treatment. She remains PEG tube dependent for 100% of her nutrition. She continues to suffer severe trismus – with her mouth opening only about 10 mm.

The swallowing problems have led to two episodes of aspiration pneumonia that occurred during late winter and early spring, 2009. The first episode needed VATS decortication and a two week in-patient stay to remove empyema.

Sadly, on July 18, 2009, she was diagnosed with distant metastatic disease with tumor found in both lungs and on a rib. While the opinions varied among physicians, the best any forecasted was a very slim chance for a five year survival. One thought life expectancy was as short as a year.

Given Linde's history of aspiration pneumonia, the hematologist oncologist at Wilmot suggested we seek an additional opinion from Dr. Everett Vokes at the University of Chicago. This same Wilmot hematologist oncologist sagely offered advice to be careful as you choose a treatment plan to balance mortality with the quality of life. He offered that by definition distant metastatic disease is not curable.

Following consultation with Dr. Vokes, Linde opted to still aggressively treat the distant mets, receiving infusions of weekly cetuximab and of tri-weekly cisplatin plus taxotere. The cetuximab is to continue for a year. The cisplatin and taxotere were to be provided for six cycles, assuming the tumors were controlled.

The trusting relationship bond Linde enjoyed with the Roswell surgeon was also found with Dr. Vokes. While encouraging, he noted there was less than a 10% chance of long term survival. His memorable words were, "Plan for the worst and be pleasantly surprised if there is a better outcome."

Linde was told side effects from the chemotherapy would include fatigue, nausea and hair loss but she still wasn't ready to give up on survival.

However, once again, the list was not exhaustive. Linde suffered both mucositis and herpes zoster.

Linde began using Dr. Vokes' protocol at Wilmot in August and happily the scan done after two cycles showed tumor shrinkage. This led to Dr. Okunieff removing four tumors using stereotactic radiosurgery. Dr. Okunieff has characterized the cancer as oligometastatic which provides us some increased hope of long term remission.

After four cycles of the cisplatin and taxotere, Linde showed signs of internal bleeding which was subsequently worked up using an EGD (Esophagogastroduodenoscopy). During this endoscopic procedure on November 2, 2009, Linde's esophagus was perforated. Fortunately, there was no sepsis. It was made very clear to us after this accident the very possible life-threatening nature of this injury.

The esophagus was perforated as the scope encountered an unanticipated stricture created by the chemoradiotherapy Linde had in the fall of 2008. The stricture measured 9 mm.

All chemotherapy was immediately suspended; the surgeons did not want the immune system compromised by the cytotoxins during esophageal healing.

Still concerned about beating the cancer, Linde asked how much cisplatin and taxotere a patient needed to effect remission and was told there was no reliable science and that four cycles may well be enough.

A workup was also done on possible dilation of the stricture. Surgeons at both Roswell and Wilmot agreed that the risk of rupture outweighed the benefits of dilation. Instead, they recommended that another round of swallowing therapy be tried.

We characterized the suspension of the chemotherapy as a chemo holiday. This respite allowed Linde to experience life somewhat as she knew it before cancer. As her energy recharged, she returned to being the very social person she is. Regardless of the long term

outcome of her treatment, this chemo holiday will be remembered for being very good. These have been moments for us to cherish.

The cisplatin and taxotere have been very rough on Linde. The fatigue has been horrible. The mucositis has been so painful that even talking hurts. While on these drugs, Linde is effectively shackled to our couch or bed, unable to do much of anything.

Adding to these problems was being feeding tube dependent and the discomfort it created for her to eat with others.

She didn't verbalize her complaints, but her unhappiness was palpable. As someone who loves her very much, it was painful for me to witness.

The chemo holiday made it clear to Linde how much her quality of life had deteriorated as she fought at all costs for survival.

The chemo holiday also provided a time for reflection and for assessing how important quality of life is.

This respite also taught both of us how important it is to cherish the moment. This is not at all a trite expression. No one knows when their last moment will occur nor how they'll feel in the time leading up to it. So it is incredibly important to make the most of each day.

It led Linde to decide the following:

No more cisplatin and taxotere. Given the misery created by these drugs and the reduction in her quality of life, Linde concluded it just isn't worth it, given her probable life expectancy. The fatigue and oral pain created by the mucositis are just too much.

She will try swallowing therapy one more time, and if it doesn't work, she will seek dilation of her esophagus regardless of complication risk. This therapy actually began on January 5. Again, she wants to partake in the social activity called eating with others.

She began weekly cetuximab again on January 11, but if it causes mucositis or herpes, it may be permanently ended. Again, a quality of life issue!

She has also made other non-medical lifestyle decisions, including going to Florida for the winter and receiving her cetuximab and swallowing therapy there. With her loss of weight, she is chronically cold. As an aside, packing her clothes normally a burdensome and tedious activity brought her obvious joy confirming the rightness of the quality of life decision.

While all decisions are surely subject to change, my experience with Linde is that she is pretty definite in her likes and dislikes. In my opinion, these are firm, well-thought-out choices.

We are trying to make the best of our time by harvesting all of the enjoyment we can from each day.

Linde did have a scan on January 13 that showed no bad news.

Our anxiety level grew as we approached this last scan and it surely will as we proceed to the next one. However, with Linde's preferences known, the course of action that will be taken is pretty clear and that by itself offers a sense of peace.

This has been quite the journey for me, too. It is not easy to be a patient advocate. In the early stages of care – diagnosis and first line treatment, the advocate is both detective and cheerleader. The detective role is largely scientific. The cheerleader is emotional and spiritual.

A friend of mine who wife is suffering metastatic bladder cancer aptly said in her early months of care that his job was to be resolutely optimistic. He would keep to himself his feelings of fear and anger. He felt by creating high expectation, high results would be achieved.

At the outset, I did the same and Linde responded as I had hoped and planned. Very frankly and unfortunately, it rendered sterile my communication with Linde. I found myself staying in a comfort zone and not really discussing feelings associated with her possibly dying.

Over time, denying my emotions led to my body rebelling. I suffered sleeplessness, tension headaches and GI issues.

As I more recently openly confronted what this horrible disease really meant for me, I started to get better. A rabbi and a psychotherapist played significant, facilitative roles in both "giving us permission" and in "scripting us" to have these very sensitive and important conversations. I began talking with her about my fears and anxiety and explicitly acknowledging Linde's pain and discomfort. I told her what she meant to me and how painful it would be if she were not here.

These were very hard dialogues because of my concern that my words would lead her to a sense of guilt, not that there was anything Linde could do to avoid or to ameliorate the situation. I also worried that Linde would interpret what I said to be uncaring or selfish.

I have been careful to not overly dwell on feelings of loneliness if not isolation that I have suffered as a result of her illness and its treatment.

This has resulted for us in lots of quiet time. Mostly, I work in my home study or Linde and I together are engaged in parallel play – reading or watching TV in the family room or bedroom. Food preparation and dining which used to be central parts of our lives have been back burnered. There has been lots of affectionate, brief complimentary comments and supportive touching. Indeed, the quality of life of the patient advocate is affected, too.

I have very candidly shared Linde's and my story because I believe we probably have it better than most. Because of my job, we have access to arguably the best medical care one can find. We also benefit from Linde's wonderful family and circle of friends who simply could not be more supportive. Yet despite this good fortune, we still have had a tough road to travel.

So what does this all mean? I offered the key takeaways earlier in this talk but please allow me to reiterate the most important thoughts from my vantage point.

1. Please recognize as you already do clinically that every patient is different and this extends to their values and emotional preferences too. Please seriously consider whether the idea suggested in the beginning of this talk – to strongly encourage the patient and their family have an early palliative care consult – makes sense.

2. Know that patient preferences will change over time as they learn about how torturous the side effects of treatment are. Rely on advocates only to aid in patient education. Patients themselves must be the decision-makers. Also make a special effort to enhance patient education about risks/side effects.

3. Please do more research and development on injury mitigation to aid in achieving not only a better quality of life but also to make it less likely that patients will shy away from treatment due to fear of possible side effects.

I had asked a friend to review of draft of this speech. She had just lost her close friend and book co-author to head and neck cancer. She confirmed the correctness of the message delivered here today but added a dimension. Because she was a patient advocate who suffered the loss of her patient, she learned first hand the power of palliative care and what it really means to have quality of life win over length of life. Importantly she said that if they had it to do all over again, they would have called for a palliative care consult sooner.

Her words were absolutely consistent with many others who have sustained similar losses. To me, this spoke volumes about the need for the profession to become more sensitive to the collateral damage being done by treatment and the need for aggressive pursuit of injury avoidance and mitigation.

You are doing life saving work. Please make sure you do all you can to make the lives that you save worth living.

Thank you.

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# PANEL 2

Patient-reported outcomes (PROs)

A patient-reported outcome (PRO) is defined as any report of the status of a patient's health condition that comes directly from a patient, without interpretation of the patient's response by a clinician or anyone else. Examples include symptoms, quality of life, treatment preferences, satisfaction with care, and medication compliance. PROs have become the gold standard for reporting on these areas, and methodological standards for developing and administering PRO instruments—and reporting data collected by such instruments—have matured over the past several years. These standards are encoded in an FDA Guidance on PROs which was released in draft form in 2006 and in final form in December 2009. The guidance specifies that PRO measures should demonstrate reliability, validity, sensitivity to score changes, and have appropriate recall periods. These properties should be demonstrated for the population of interest in any given trial. One area in which PROs are not yet standard is adverse event reporting. Currently, clinicians report adverse events in clinical trials, including symptom adverse events like nausea, fatigue, or depression. But there is abundant evidence suggesting that clinicians underestimate the frequency and severity of patients' adverse symptoms, and therefore the current model likely under-represents the true toxicity burden of interventions studied in clinical trials. In NCI-sponsored trials specifically, the standard lexicon used to report adverse events is the Common Terminology Criteria for Adverse Events (CTCAE), which is an entirely clinician-reported tool including the 10% of its items which represent symptoms. Therefore, in 2008, the NCI initiated the PRO-CTCAE project to create patient versions of those symptom items. To date, 77 symptoms of the CTCAE have been converted to PRO-CTCAE items, which are currently undergoing validation.

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## ART-RIM Workshop
## National Cancer institute

Ethan Basch, MD, MSc
Memorial Sloan-Kettering Cancer Center

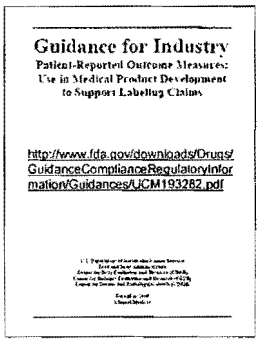
**Patient-reported Outcomes (PROs)**

January 22, 2010

## Definition

"A PRO is any report of the status of a patient's health condition that comes directly from the patient, without interpretation of the patient's response by a clinician or anyone else."

- Final FDA PRO Guidance (December 2009)


Guidance for Industry
Patient-Reported Outcome Measures: Use in Medical Product Development to Support Labeling Claims

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM193282.pdf

## Examples

- Symptoms
  - Severity, frequency, interference, bother, etc.
- Quality of life
  - Various domains
- Subjective impressions of improvement/change
- Treatment preferences
- Satisfaction with care
- Compliance with treatment

## Standards

- Rigorous standards for development, administration, analysis, and reporting of patient-reported data have emerged, and are codified in the FD A Guidance
  - Technically only apply when measuring effects of treatment with the intention of making a labeling claim, but have been widely accepted beyond the regulatory setting
  - Nonetheless, poorly designed measures are still common in protocols and publications

## Scrutiny

- Questions for patients should not simply be "made up" and adminis tered at occasional or inconsistent intervals
- Concepts that are best known by patients should not be reported by clinicians

## Considerations in *Developing or Selecting* a PRO Measure

Measurement properties of instruments

- Reliability
  - Test-retest
  - Internal consistency
- Validity
  - Content validity (qualitative)
  - Construct validity (discriminant)
- Ability to detect change
- Recall period

## Considerations when *Administering* a PRO Measure in a Trial

Population issues
- Validity, literacy, language, cognitive abilities, PS

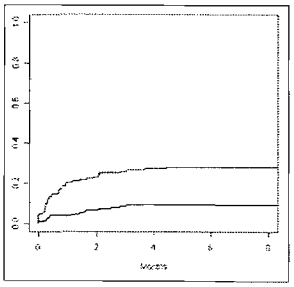
Study design issues
- What concepts to measure, parsimony
- Frequency and duration of administration
- Method and location of administration

Missing data
- Most ill and most well patients?
- Backup data collection methods?

## PROs for Measuring *Adverse Symptoms*

- Standard approach to measuring AEs in NCI-sponsored clinical trials: CTCAE
  - CTCAE v4: >800 items; ~10% are "symptoms"
- CTCAE items are reported by *clinicians*
  - But clinicians underestimate the frequency and severity of patient symptoms
  - Therefore, with clinician-only reporting, we have an incomplete picture of toxicity

## Patient vs. Clinician Reporting

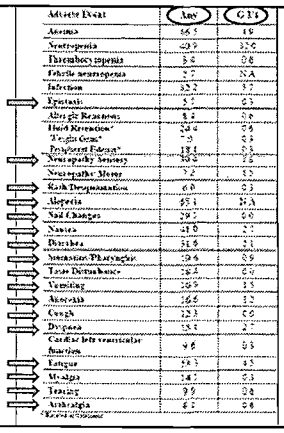



## Adverse Events in Current Labels

- Almost half are symptoms

| Indication | # of U.S. Approved Drug Labels | Average # of AEs per Label | Total # of Unique AEs across Labels | Proportion of AEs which Are Symptoms |
|---|---|---|---|---|
| Asthma | 35 | 54 | 368 | 49% (180/368) |
| Breast Cancer | 32 | 78 | 616 | 36% (223/616) |
| GERD | 38 | 115 | 472 | 45% (213/472) |
| Hyperlipidemia | 28 | 82 | 365 | 43% (158/365) |
| Osteoarthritis | 39 | 94 | 684 | 41% (278/684) |

## Docetaxel Drug Label



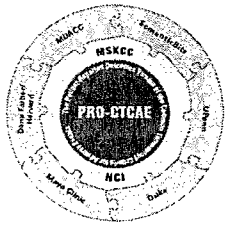
*Data from "Tax 327" pivotal trial*

**NCI contract to develop a PRO version of the CTCAE**

- Initiated 10/08

**Development of the Patient-Reported Outcomes version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE)**

NCI HHSN261200800043C



## Mission of PRO-CTCAE Initiative

- Employ rigorous scientific methods to create a system for patient self-reporting of adverse symptoms in cancer trials, which is widely accepted and used; generates useful data for investigators, regulators, clinicians and patients; and is compatible with existing adverse event reporting systems

## PRO-CTCAE Scope

- Create PRO-CTCAE adverse symptom items
- Evaluate measurement properties of items
- Build electronic administration platform
- Assess feasibility

Nine interdisciplinary task committees assembled

- Including NCI and FDA representatives

## 77 CTCAE Symptoms Identified

| | | | | | | |
|---|---|---|---|---|---|---|
| Abdominal pain | Depression | Edema – Limb | Hot flashes | Myalgia | Rash | Urticaria |
| Acne | Dermatitis (Radiation) | Ejaculatory dysfunction | Hyper-pigmentation | Nail changes | Rash: Hand-Foot | Vaginal discharge |
| Alopecia/hair loss | Diarrhea | Epistaxis (Nosebleeds) | Hypohidrosis | Nausea | Rigor/chills | Vaginal dryness |
| Amenorrhea | Distension/ bloating, abdominal | Erectile dysfunction | Incontinence, anal | Neuropathy-sensory | Skin Breakdown | Vision – blurred |
| Anorexia | Dizziness | Fatigue | Incontinence, urinary | Odor | Solar | Voice changes |
| Anxiety | Dry mouth/ xerostomia | Flashing lights | Injection site reaction | Orgasmic dysfunction | Sweating diaphoresis | Vomiting |
| Arthralgia (joint pain) | Dry skin | Flatulence | Insomnia | Pain | Taste alteration | Watery eye |
| Bronchospasm, wheezing | Dysarcevnia | Flushes | Irregular menses | Painful urination | Tinnitus | Decreased concentration |
| Cheilitis | Dysphagia | Gynecomastia | Libido | Palpitations | Tremor | Depression 2 |
| Constipation | Dyspnea | Heartburn/ dyspepsia | Memory impairment | Photo-sensitivity | Urinary frequency | Nail changes 2 (color) |
| Cough | Easy bruising | Hiccoughs | Mucositis/ stomatitis | Pruritus/ itching | Urine color change | Nail changes 3 (shape) |

## Possible Attributes of Each Symptom

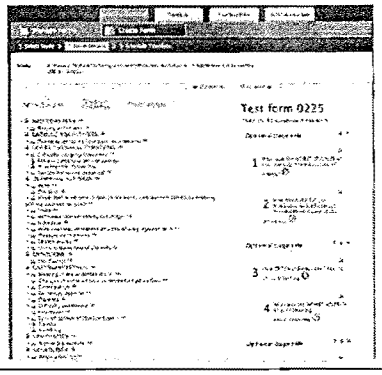
- Frequency
- Severity
- Interference with usual activities
- Present/Not present

- Separate items for each attribute
  - Between 1 and 3 attribute items per symptom
  - Selected based on attributes included in original CTCAE items, and nature of each symptom
  - 122 total items representing the 77 symptoms

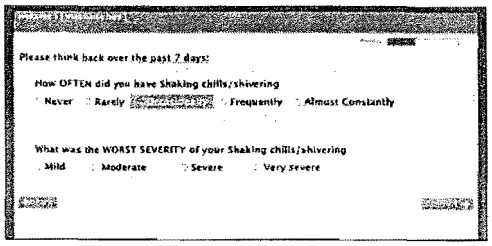
## Methodological Development

- Content validity study
  - Cognitive interviews
- Measurement properties study
  - Validity, reliability, sensitivity, recall
- Platform "usability" study
- Feasibility study

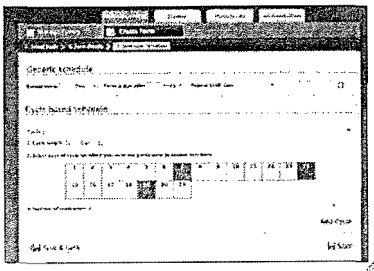
## Platform: Form Builder



## Platform: Patient Interface




## Platform: Study Calendar



## Summary

- Patient self-reporting is the gold standard for symptom assessment
- Guidance for developing and administering PRO instruments is available in the FDA document
- The PRO-CTCAE provides a lexicon of adverse symptom items which are being developed in keeping with rigorous methodological criteria

## Col. Patricia Lillis-Hearne, M.D., AFRRI, USUHS

(No summary received)

Gary R. Morrow, PhD, MS

A key goal of this workshop is to:

"iron out how to efficiently move the most promising agents into appropriate clinical trials"

Several things may affect that efficiency. Experience from over thirty five years of conducting clinical trials has shown the importance of minimizing delay and confusion as essential to the completion of multicenter clinical trials. Discussions during a 1982 conference to explore adding quality of life measures to cancer clinical trials and discussions in 2000 where a pharmaceutical company sought assistance in measuring fatigue as a patient reported outcome highlight potential delays in agreeing on appropriate outcome measures. At the end of both sets of discussions, representatives of the FDA said that a document with guidance on appropriate measures was in progress. It was released last month. Recent experiences obtaining an IND to study Curcumin included 57 different contacts with the FDA over 412 days. This does not minimize delay and confusion.

Resources in the NCI sponsored Community Clinical Oncology Program provide appropriate clinical trials with existing, proven infrastructure.

Centering discussion of this key goal on the following four questions could help meet the goal:

1) What is appropriate FDA review expertise for this research?
2) Where is it found administratively?
3) How can mutually beneficial relationships be promoted?
4) Is there a way to have all this be more timely?

Gary R. Morrow, Ph.D., M.S.

Associate Director for Community Research
Coordinator of Research Training

James P. Wilmot Cancer Center

University of Rochester

Email: Gary_Morrow@URMC.Rochester.edu

## The key goals of this workshop are to:

1) recommend best (preclinical) practices for efficiently evaluating and developing agents in the CMCR pipeline for possible applications in cancer patients

2) iron out how to efficiently move the most promising agents into appropriate clinical trials

3) develop a summary "position paper" to be published

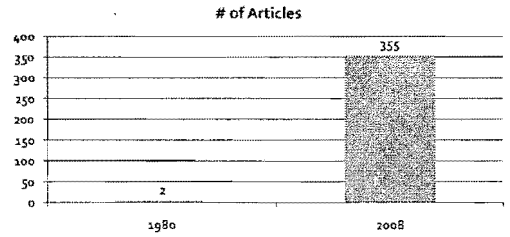
## Three (hopefully idiosyncratic) stories of reduced efficiency that led to increased delay and confusion

- Quality of life (mid 1980's)
- Fatigue (2000)
- Curcumin (2006)

## Key to clinical trials:

- minimize DELAY and CONFUSION

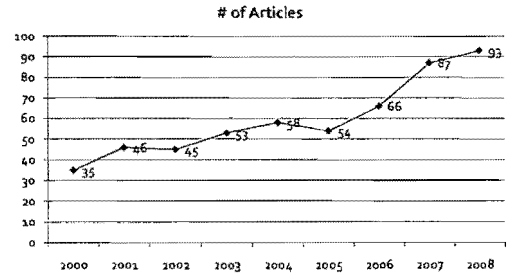
## Number of Articles in Pub Med with 'Cancer' and 'Quality of Life' in Titles




QOL and cancer in title 1980 = 2 = .05% of articles with cancer in title (cancer alone = 3966)
QOL and cancer 2008 = 355 = 1.2% of articles with cancer in title (cancer alone = 29940)

## "The Patron Saint of Clinical Trials"

## Number of Articles in Pub Med with 'Cancer' and 'Fatigue' in title by Year



## Policy Statement

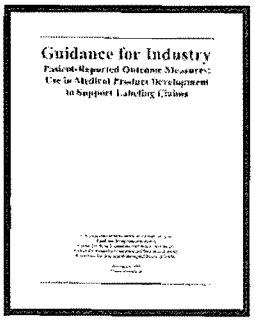
## The Rest of the Story: the cast of players

**Involved :**

- 9 FDA staff
- 4 FDA divisions
- 1 FDA Ombudsman
- 1FDA Chief
- 1 US Congresswoman
- 1 US Senator
- 8 University of Rochester people

And 57 separate contacts over the 412 days

## Better later than never?


Guidance for Industry

Patient-Reported Outcome Measures: Use in Medical Product Development to Support Labeling Claims

## Community Clinical Oncology Program: appropriate clinical trials

***What is a CCOP Research Base?***

- An NCI-designated Cancer Center or Cooperative Group
- Funded by a Peer- Reviewed Cooperative Agreement
- Develop and Conduct Cancer Prevention and Control Clinical Trials
- Supports Development of Cancer Prevention Science

## The Curcumin Soap Opera: more delay and confusion

- June 30, 2006 IND submitted
- August 4, 2006 IND approved
- August 31, 2006 "complete clinical hold"

- July 25, 2007 "proceed"
- July 26, 2007 "hold"
- August 16, 2007 "approved"

## Community Clinical Oncology Program

**CCOP Research Bases: Cooperative Groups**

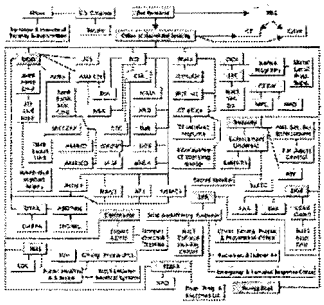
- Children's Oncology Group
- Cancer and Leukemia Group B
- Eastern Cooperative Oncology Group
- North Central Cancer Treatment Group
- Radiation Therapy Oncology Group
- Southwest Oncology Group
- National Surgical Adjuvant Breast & Bowel Project
- Gynecologic Oncology Group

## Community Clinical Oncology Program

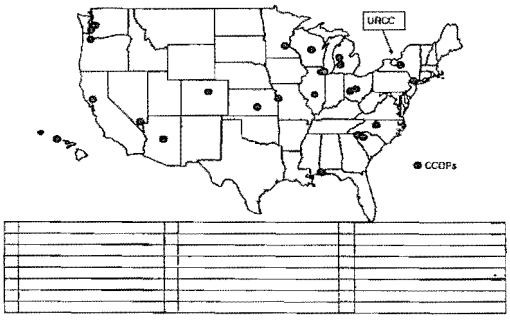
CCOP Research Bases: Cancer Centers

- H. Lee Moffitt Cancer Center
- M.D. Anderson Cancer Center
- University of Rochester Cancer Center
- Wake Forest University Cancer Center

## New, Improved CCOP Protocol review



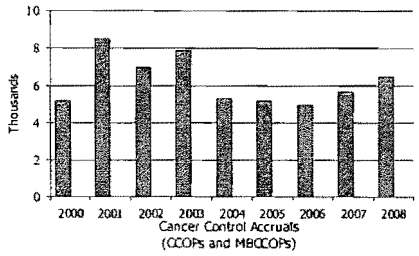
## UNIVERSITY OF ROCHESTER CANCER CENTER COMMUNITY CLINICAL ONCOLOGY RESEARCH BASE MAP OF AFFILIATES (2009)



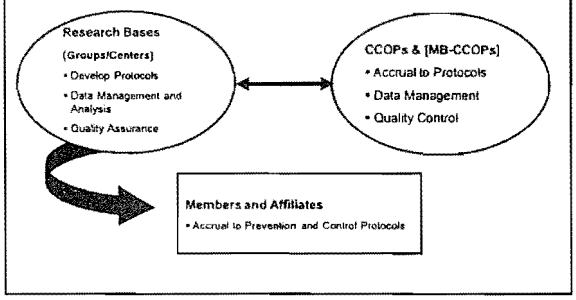

## How to iron out how to efficiently move the most promising agents into appropriate clinical trials:

- What is appropriate FDA review expertise for this research?
- Where is it found administratively?
- How can mutually beneficial collaborative relationships be promoted?
- Is there a way to have this all be more timely?

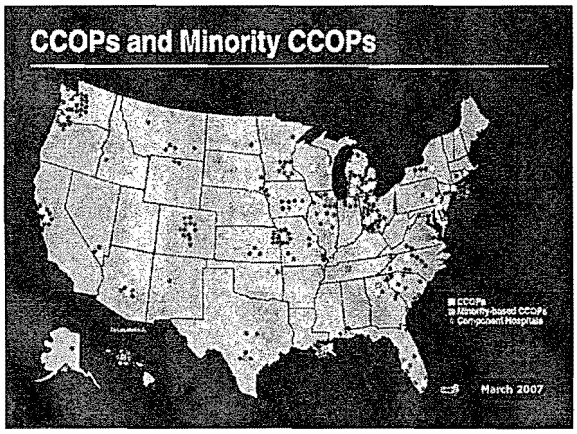
## Community Clinical Oncology Program Accruals




## CCOP Organizational Relationships




## CCOPs and Minority CCOPs




# PANEL 3

Ian Stratford, M.D., Ph.D.

## Drugs for combining with radiotherapy: Drug targets, the pipeline and evaluation

Combining standard chemotherapy with radiation for the treatment of various cancers has developed, in the main, empirically. Although subsequently there has been some elegant attempts to explain both the positive and negative clinical findings (see e.g. Bentzen *et al* Nature Clinical Practice Oncology, 2007, 4, 172-180). In the review by Bentzen *et al*, they identified five distinct mechanisms by which drugs and radiation could interact: Cytotoxic enhancement, temporal modulation, biological cooperation, special cooperation and normal tissue protection. In formulating these mechanisms the authors took account of the 5Rs of radiotherapy. However, with the plethora of new molecular targets that have been identified and validated, and the variety of drugs that have been developed to hit these targets, there now an urgent need to consider how such agents can be best integrated with radiotherapy.

Successful integration will require appropriate and robust evaluation both pre-clinically and in early phase clinical trial. However in designing the evaluation procedure there is major need to have an understanding of how the new agents may impact on any of the 5Rs. In addition there is always the question "does radiation effect the expression and function of the target?" From a pre-clinical stand-point there is a requirement for both in vitro and in vivo evaluations. These will be discussed and examples given to show how the underlying mechanisms of the drug/radiation interaction(s) can profoundly effect outcome.

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# Paul Okunieff, M.D.

(Slides only - no summary)

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Chairman, College of Medicine
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# Radiation Countermeasures: Interfacing with Clinical Oncology

**Paul Okunieff, MD**
*CMCR Steering Committee Chair*
*CBARMFI (Rochester) Principal Investigator*

## OVERVIEW OF PROGRESS SINCE 2005

### Can radiation side effects be mitigated?

Many new agents and mechanisms *(there were none in 2005)*
Improved supportive measures *(those used before 2005 might have been detrimental)*
New industry base *(mostly micropharma)*

### Can biodosimetry be used to perform mass screening?

Many new technologies for genotoxicity measurement
Many new technologies and biological markers for metabolic response
*These have implications for following of cancer patients*

## FDA Requirements:

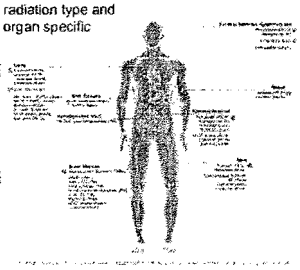
1. Mitigation in at least 1 animal model (logically requiring primates)
2. Sufficient benefit to be worthwhile in animal model of specific scenario
3. Proven mechanism of action
4. Similarity of action between species tested and man
5. Safety in an otherwise healthy population

The money spent so far in the CMCR and other NIAID, DARPA, and BARDA objectives have been rewarded by substantial practical progress but no approved agents. Agencies looking for a better partnership with FDA

## State of CMCR Drug Development:

**CMCR Network**
Agent Development Status

The needed agents are time, dose, radiation type and organ specific



**PHEMCE Strategy**
Most Grants/Contracts for Acutely Lethal Events: Many of the official scenarios indicate that the likely risks for radiation exposure are for ***inhalational, cutaneous, and partial body exposures leading to delayed death***

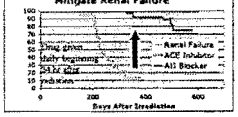
**Over-avoidance of Cancer Patient Testing:**
**Survivorship research**

**Long-term grants/contracts:**
**Longer term product development grants are only recently aimed at industry that grows from academia**

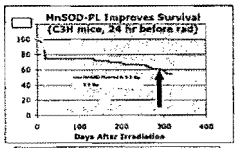
## Implications of FDA Requirements:

1. Low hanging fruit: agents already know to be safe and already subject to substantial human testing
2. Creative agents a major challenge: mechanisms not likely to be approved, safety unknown
3. Challenge regarding mechanism: mechanism is not a critical part of most FDA approvals, it is just supportive for most approvals.

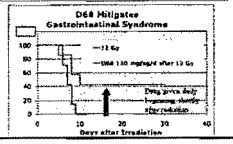
Potential for agents to become better understood and validated if used in a cancer population. The FDA's acceptance of toxicity is naturally higher in cancer patients than it would be in a healthy population




**Medical College of Wisconsin (J Moulder)**
Renal failure begins 6 mo after irradiation.
Angiotensin II blockers reduce or least greatly delay that lethal renal failure.




**University of Pittsburgh (J Greenberger)**
Gene therapies in human clinical trials for cancer patients are underway.




**University of Rochester (P Okunieff)**
This CMCR agent mitigated GI syndrome with survival up to 16 Gy (Control lethal dose ($LD_{50}$) = 8 Gy).

## Range of Devices and Agents that Need to be Developed

**Triage:** exposed vs. not-exposed
- Proteomic
- Genomic/ genotoxicity
- Metabolomic

Simple (desktop) vs. Advanced biomology

**Dosimetry:** Actual physical dose received (whole vs. partial body)
- Cytotoxicity assays
- Physical chemistry methods (free electrons in teeth, nails)

**Mitigation:** Agents given after exposure that prevent late deaths
- Example: delayed death due to lung or renal failure

**Treatment:** Agents that reduce severity of existing problems
- Antibiotics, growth factors

**Epidemiology:** Tools to allow long-term evaluation of survivors
- Critical for agent development and validation but requires useful interventions and technologies as defined above

## Steering Committee Strategy:

**MAJOR STRATEGIC POINTS**

1. There are many very promising translational research projects in development for agents and instruments to deal with radiological events including: (1) biodosimetry, (2) mitigation, and (3) treatment. There were next to none in 2005
2. The Stockpile does not yet have many fully satisfactory agents or instruments for radiological threats.
3. We believe the FDA should allow cancer patients to be considered a "primate" model and peaceful uses of the technology

We don't want to have to say again in 2015 that the money spent so far in the CMCR and other NIAID, DARPA, and BARDA objectives have been rewarded by substantial practical progress… but no products

**Mitigation of Chronic Radiation Injuries by ACE Inhibitors and AII Blockers**

**John Moulder, Ph.D.**
Center for Medical Countermeasures Against Radiological Terrorism
Medical College of Wisconsin

Suppression of the renin-angiotensin system with angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (AII blockers) is of clear benefit in the mitigation of experimental radiation nephropathy *[1,2]*; and there is evidence for their efficacy in the mitigation of clinical radiation nephropathy *[3]*. Both ACE inhibitors and AII blockers have efficacy in the mitigation of experimental radiation-induced lung *[4,5]* and CNS injury *[6,7]*. More recently, AII blockers have been shown to mitigate TBI-induced cardiac injury *[8]*, and ACE inhibitors have been shown to mitigate both radiation-induced cutaneous injury and a combined cutaneous radiation-wound injury (Z. Lazarova, personal communication). The mechanism of the effect is unclear, but it does not appear to be due to radiation-induced upregulation of the renin-angiotensin system *[9-11]*. The experimental efficacy of these agents at clinically-relevant drug doses, combined with their wide-spread clinical use for other types of injury, makes use of these agents for the mitigation of radiation injuries feasible.

1. EP Cohen, MM Joines, JE Moulder: Prevention and treatment of radiation injuries - The role of the renin-angiotensin system. In *Late Effects of Cancer Treatment on Normal Tissues* (P Rubin, LS Constine, LB Mark, P Okunieff, Eds.), pp. 69-76. Springer-Verlag, Heidelberg, 2008.
2. JE Moulder, EP Cohen: Future strategies for mitigation and treatment of chronic radiation-induced normal tissue injury. *Sem Rad Onc* **17**, 141-148 (2007).
3. EP Cohen, AA Irving, WR Drobyski, JP Klein, J Passweg, J Talano et al.: Captopril to mitigate chronic renal failure after hematopoietic stem cell transplantation: a randomized controlled trial. *Int J Radiat Oncol Biol Phys* **70**, 1546-1551 (2008).
4. A Molteni, JE Moulder, EP Cohen, WF Ward, BL Fish, JM Taylor et al.: Control of radiation-induced pneumopathy and lung fibrosis by angiotensin converting enzyme inhibitors and an angiotensin II type 1 receptor blocker. *Int J Radiat Biol* **76**, 523-532 (2000).
5. SN Ghosh, R Zhang, BL Fish, VA Semenenko, XA Li, JE Moulder et al.: Renin-angiotensin system suppression mitigates experimental radiation pneumonitis *Int J Radiat Oncol Biol Phys* **75**, 1528-1536 (2009).
6. S Ryu, A Kolozsvary, KA Jenrow, SL Brown, JH Kim: Mitigation of radiation-induced optic neuropathy in rats by ACE inhibitor ramipril: importance of ramipril dose and treatment time. *J Neuro-Oncol* **82**, 119-124 (2007).
7. ME Robbins, V Payne, E Tommasi, DI Diz, FC Hsu, WR Brown et al.: The $AT_1$ receptor antagonist, L-158,809, prevents or ameliorates fractionated whole-brain irradiation-induced cognitive impairment. *Int J Radiat Oncol Biol Phys* **73**, 499-505 (2009).
8. JE Baker, BL Fish, JE Moulder: Total body irradiation-induced increase in risk factors for cardiovascular disease are mitigated by post irradiation treatment with Losartan and Curcumin. In *55th Annual Meeting of the Radiation Research Society*, Savannah, 2009.
9. EP Cohen, BL Fish, M Sharma, XA Li, JE Moulder: Role of the angiotensin II type-2 receptor in radiation nephropathy. *Trans Res* **150**, 106-115 (2007).
10. EP Cohen, BL Fish, JE Moulder: The renin-angiotensin system in experimental radiation nephropathy. *J Lab Clin Med* **139**, 251-257 (2002).
11. MEC Robbins, D Campling, M Rezvani, SJ Golding, JW Hopewell: Radiation nephropathy in mature pigs following the irradiation of both kidneys. *Int J Radiat Biol* **56**, 83-98 (1989).

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*Summary of Presentation at Meeting on Advanced Radiation Therapy (ART) Radiation Injury Mitigation (RIM), Monday January 25, 2010.*

# Somatostatin Analogs: Effective Mitigation of Intestinal Injury in Clinical Radiation Therapy and Radiological/Nuclear Terrorism Scenarios

**Martin Hauer-Jensen, MD, PhD, FACS**

University of Arkansas for Medical Sciences and Central Arkansas Veterans Healthcare System, Little Rock, AR, USA

Intestinal radiation toxicity is a major dose-limiting factor during radiation therapy of abdominal and pelvic tumors. The severity of intestinal injury is also a critical determinant of survival after whole body radiation exposure in nuclear accidents or radiological terrorism scenarios.

The contents of the bowel lumen, notably the exocrine pancreatic secretions, exert a major influence on the development of intestinal radiation toxicity. Hence, extensive studies in dogs and rodents have demonstrated that surgical removal of the pancreas, pancreatic duct-occlusion, or inhibition of pancreatic enzymes in the bowel lumen reduce lethality after abdominal irradiation and ameliorates structural and functional toxicity after localized intestinal irradiation.

In humans, effective inhibition of exocrine pancreatic secretions can be achieved by the use of somatostatin analogs. Somatostatin analogs are safe, free of side effects and drug interactions, and lack tumor-protective properties. Moreover, because somatostatin analogs strongly inhibit gastrointestinal motility and secretion, they are also used clinically to treat severe diarrhea associated with cancer therapy.

Our laboratory has performed a series of studies to test the efficacy of somatostatin analogs as gastrointestinal radiation response modifiers. Studies in a clinically relevant rat model with fractionated, localized intestinal irradiation demonstrated that somatostatin analogs confer effective protection against radiation mucositis, reduce the development of delayed bowel fibrosis, and prevent excessive activation of proteinase-activated receptor 2 (PAR2), a receptor involved in gastrointestinal inflammation and nociception. Subsequent clinical studies performed by others confirm that somatostatin analogs ameliorate symptoms of acute mucosal injury during radiation therapy in humans and reduce treatment interruptions. Most remarkably, studies with whole body irradiation in mice demonstrate that SOM230, a novel somatostatin analog, confers 50-60% lethality protection (dose reduction factor 1.2), regardless whether drug administration begins prior to irradiation or as late as 48 hrs after radiation exposure.

In conclusion, somatostatin analogs are uniquely suited as enteroprotective agents because of their therapeutic efficacy, safety of use, lack of tumor protection, ease of stockpiling and administration, and remarkably wide "time window". The following should be considered indications for the use of somatostatin analogs 1) cancer patients undergoing radiation therapy; 2) first responders and cleanup personnel after radiation accidents or attacks; and 3) post-exposure casualties in the radiological/nuclear emergency setting.

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**George E. Georges, M.D., Fred Hutchinson Cancer Research Center**
and University of Washington, Seattle

Hematopoietic stem cells (HSC) survive and reconstitute hematopoiesis after 8 Gy total body irradiation (TBI) in dogs given intensive supportive care and cytokine treatment.

Hematopoietic cells are highly sensitive to TBI and their loss after radiation exposure results in lethal infections. However it appears that HSCs are more radiation resistant than committed hematopoietic progenitor cells. The difficulty in surviving the hematopoietic syndrome with prolonged pancytopenia after high dose of TBI has made it experimentally challenging to determine if HSCs survive high dose TBI in large animals that are not in a pathogen-free environment. We asked if intensive supportive care and cytokine treatment after high dose TBI would permit survival and recovery of endogenous hematopoiesis without requiring HSC transplantation in the well-established dog model. Historical results showed that after 4 Gy TBI and limited supportive care, only 1 of 28 dogs survived. The intensive supportive care regimen given after TBI included an antibiotic use algorithm for empiric treatment of prolonged neutropenia and fever. Blood transfusion support was given for platelet counts < $6x10^9$/L and hematocrit < 24%. With intensive supportive care, we observed uniform survival and endogenous hematopoietic recovery in dogs following 5, 6, and 7 Gy TBI. The LD50 at 100 days was 8 Gy TBI. Cytokine treatment consisted of either granulocyte colony stimulating factor (G-CSF) alone or combined G-CSF and flt-3 ligand, (FL). Treatment with cytokines started 2 hours after TBI and continued until absolute neutrophil count (ANC)>1000/μL. Cytokine treatment did not improve survival compared to recipients of intensive supportive care alone, but it significantly decreased the duration of intensive supportive care. For all cohorts receiving cytokines, ANC recovery was more rapid compared with supportive care alone ($p$<0.002). In addition, FL recipients had a more rapid recovery of platelet counts with reduced transfusion needs compared to supportive care alone or G-CSF treatment. Follow-up of dogs to 2.5 years after TBI showed sustained hematopoiesis and immune reconstitution without leukemia or evolution of significant clonal cytogenetic abnormalities. The results show that HSCs survive after 8 Gy TBI, intensive supportive care is sufficient to permit survival after 8 Gy TBI, and that the cytokine combination G-CSF/FL promotes more rapid recovery of ANC and platelets. The results are highly relevant for the treatment of victims of terrorist or accidental radiation exposure.

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Nelson J. Chao, M.D., MBA, Professor of Medicine and Immunology,
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Human Growth Hormone as a Radiation Mitigator

We studied the ability of recombinant human growth hormone (rhGH) to mitigate against radiation injury in mice and nonhuman primates. BALB/c mice were irradiated with 7.5 Gy and treated post-irradiation with rhGH intravenously at a once daily dose of 20 μg/dose for 35 days. rhGH protected 17 out of 28 mice (60.7%) from lethal irradiation while only 3 out of 28 mice (10.7%) survived in the saline control group. A shorter course of 5 days of rhGH post-irradiation produced similar results. Compared with the saline control group, treatment with rhGH on irradiated BALB/c mice significantly accelerated overall hematopoietic recovery. Specifically, the recovery of total white cells, CD4 and CD8 T cell subsets, B cells, NK cells and especially platelets post radiation exposure were significantly accelerated in the rhGH-treated mice. Moreover, treatment with rhGH increased the frequency of hematopoietic stem/progenitor cells as measured by flow cytometry and colony forming unit assays in bone marrow harvested at day 14 after irradiation, suggesting the effects of rhGH are at the hematopoietic stem/progenitor level. rhGH mediated the hematopoietic effects primarily through their niches. Similar data with rhGH were also observed following 2 Gy sublethal irradiation of nonhuman primates. Our data demonstrate that rhGH promotes hematopoietic engraftment and immune recovery post the exposure of ionizing radiation and mitigates against the mortality from lethal irradiation even when administered after exposure.

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# PANEL 4

Julie L. Ryan, PhD, MPH, Assistant Professor, Departments of Dermatology & Radiation Oncology, University of Rochester Medical Center

# Preclinical Guidelines: Development of Radioprotective/Mitigative Agents

The radioprotection/mitigation development program will identify agents that protect or mitigate radiation-induced epithelial, mucosal, and neurocognitive damage, improve survivorship, quality of life, and palliative care, and potentially prevent secondary or recurrent cancers. The primary objective of the radioprotection/mitigation development program is the development of agents which selectively protect normal tissues (not tumors) against ionizing radiation. Secondarily, the development of these agents will improve patient quality of life through the prevention and/or reduction of radiation treatment-related toxicities. Regardless of the time of administration, agents with the most promise will be evaluated in this program designed to develop the agents that most effectively protect normal tissues, but not tumors, against ionizing radiation. Additionally, promising agents will be further evaluated to determine their ability to prevent late radiation effects and cancer recurrence. We have developed preclinical guidelines to facilitate that development and transition of radioprotective/mitigative agents into clinical trials. Candidate agents, from various sources such as NIAID's CMCR, will be selected based on one or more of the following criteria: 1) candidate agent protects normal tissue from radiation damage; 2) candidate agent protects specific normal tissue from radiation; and/or 3) candidate agent does not protect tumors from radiation. Selected candidate agents will be further evaluated under three additional stages of development: a) Stage I: Toxicity and Maximum Tolerated Dose; b) Stage II: Radiation Protection/Mitigation Activity; and c) Stage III: Drug Evaluation, Production, and Formulation for Clinical Trials. An agent must pass each stage of development before progressing to the next stage. These preclinical guidelines are designed to aid, not impede, development of radioprotective/mitigative agents. Therefore, it is essential to determine the minimal and acceptable data and assays required for successful advancement of this field. Additionally, alternative funding options for this research need to be explored due to the limitations of CMCR.

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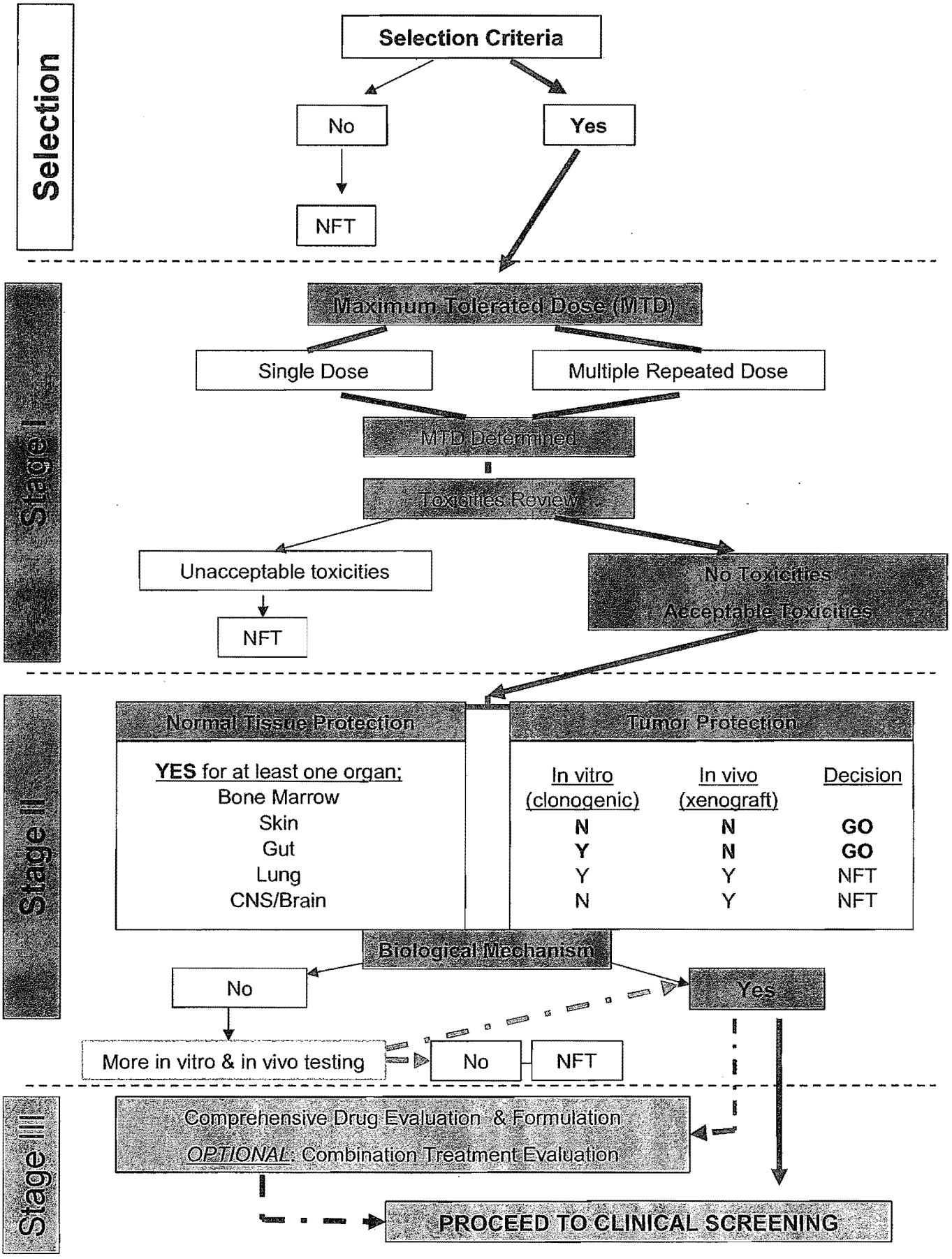
## Radioprotector/mitigator Screening Program



Stephen L. Brown, Ph.D.

Pharmacological Agents that Reduce Radiation Injury to Normal Tissue and Do Not Reduce Anti-tumor Effect of Radiation

Preclinical studies have identified a number of compounds that at doses which are known to be safe and achievable in humans have been shown in animal models to reduce normal tissues radiation injury and demonstrate anti-cancer effects. Promising compounds are grouped according to their time of administration. Those with the greatest effect when given hours before or after radiation probably work by enhancing or interfering with DNA repair of normal tissue or tumor, respectively. It is hypothesized that the differential effect on tumor and normal tissue DNA repair enhances or interferes with apoptosis of tumor or normal tissues, respectively. An example of the first group of compounds is HDAC inhibitors. The second group of agents exhibits their effect on normal tissue days and weeks after radiation probably by reducing inflammation that would lead to subsequent injury. Example agents include ACE inhibitors and statins. At least some ACE inhibitors demonstrate a cytostatic effect on proliferating cells even in the absence of radiation. In general, the second group of agents exhibits a small amount of radio-sensitization in tumors in sharp contrast to their effect on irradiated normal tissues. A third group of agents, those which are best applied weeks or months after radiation, include regenerative strategies such as stem cell therapy. Research in this area is in its early stages and many questions regarding the potential of this approach remain unanswered. For example, the inadvertent effect on a tumor's stem cell population of approaches designed to enhance the normal tissue endogenous stem cell population are unknown. In conclusion, opportunities currently exist to 1) use pharmacological agents (FDA approved for other indications) with anti-cancer intent that if timed properly may improve normal tissue response and to 2) use pharmacological agents (FDA approved for other indications) which reduce normal tissue injury and if timed properly will also exhibit anti-cancer activity. Finally, there is a need for future pre-clinical work to further quantify the effects of radiation injury on normal tissues (HDAC inhibitors), tumor models (ACE inhibitors and statins) and to elucidate the mechanisms of differential effects on tumor/normal tissue for these promising compounds.

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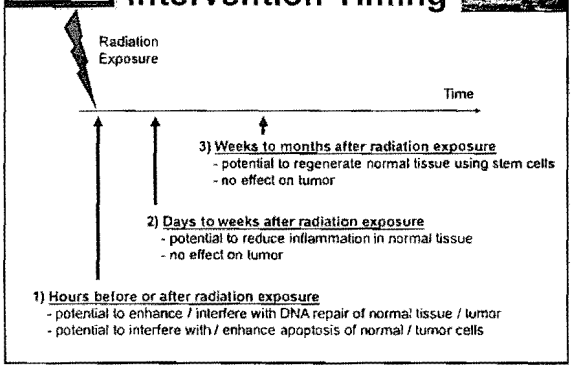
# Preclinical studies showing protection of normal tissues and lack of protection for tumors

Stephen Brown
Henry Ford Hospital, Detroit MI

## Intervention Timing




## Criteria for Drug Selection

- FDA approved
- Potential as an anti-cancer approach but may also mitigate normal tissue injury
- Potential to reduce normal tissue injury for other indications (ACE inhibitor – heart, statin - brain)

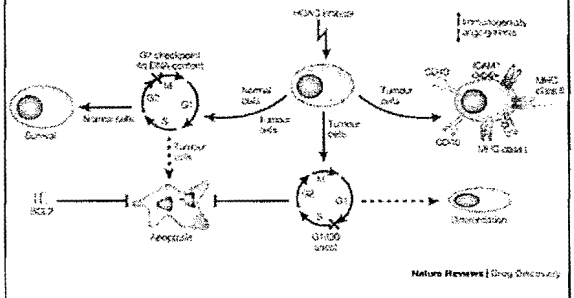
## Outline

- Introduction
- Potential pharmacological approaches
  - Example 1: HDAC inhibitor
  - Example 2: ACE inhibitor
  - Other promising approaches
- Conclusions
- Unanswered Questions / Future Work

## HDAC inhibitor radiosensitize cancer cells *in vitro* and *in vivo*

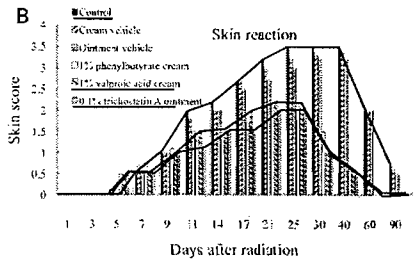
- Mira Jung, Georgetown University
- Philip Tofilon and Kevin Camphausen, NCI

## Effect of HDAC inhibitors on Tumor / Normal Cells



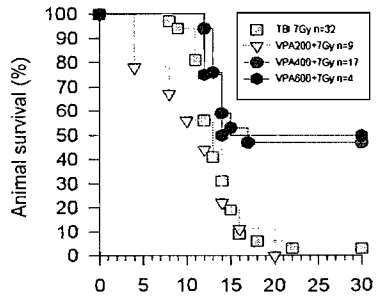
Johnstone RW. Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nature Reviews Drug Discovery* 1: 287-299 (April 2002).

## HDAC inhibitors protect normal tissue from radiation injury

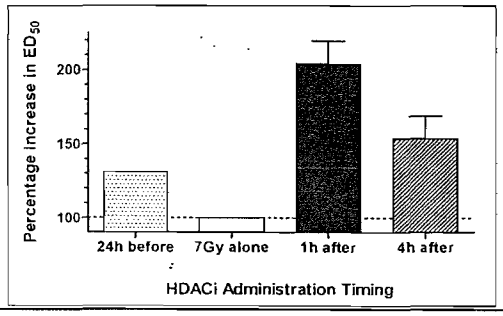



Chung YL, Wang AJ, Yao LF. Antitumor histone deacetylase inhibitors suppress cutaneous radiation syndrome: Implications for increasing therapeutic gain in cancer radiotherapy. Mol Cancer Ther. 3: 317-325 (2004).

## HDAC inhibitors




## Effect of HDAC inhibitor Sequence and Timing



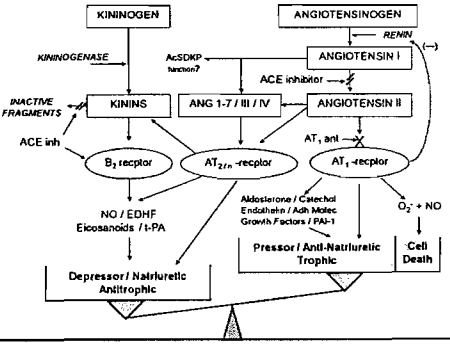

## ACE inhibitors mitigate radiation effects on multiple-organs and tissues

**(e.g. lung, kidney, skin, CNS, etc).**

## *ACE Inhibition Reduces Radiation Injury*

- W. Ward showed Captopril protected radiation pneumonitis (1986).
- J. Moulder showed ACEi protected radiation nephropathy (1993).
- J.H. Kim and co-workers showed ACEi, ramipril, mitigated radiation optic neuropathy (2003) and skin (2007).
- Z. Lazarova and J. Moulder showed ACEi mitigated combined radiation/trauma with skin (2009).
- M. Medhora and J. Moulder showed ACEi mitigated radiation nephropathy (2009).

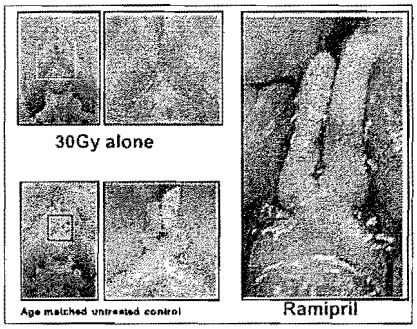
## ACE inhibitor



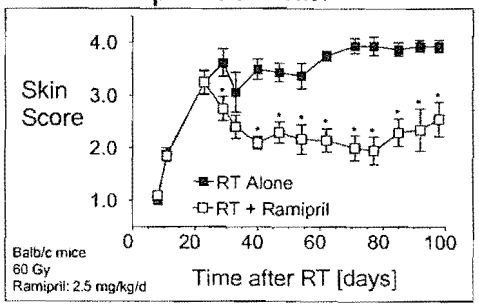
## *ACEi as Cardio-Protectors*

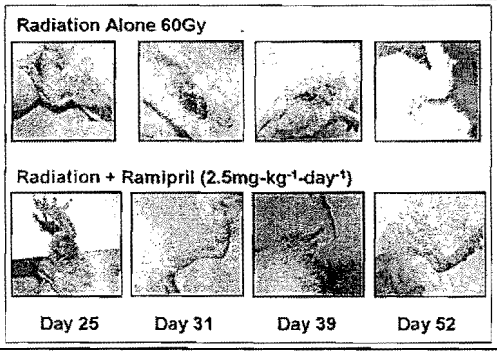
- Reduction of AT2 and blockade of AT1 receptor increase cardiac protection.
- Aspartyl tetra-peptide and kinins (increases after ACEi) have cardio-protective effects.

## Radiation-induced optic neuropathy

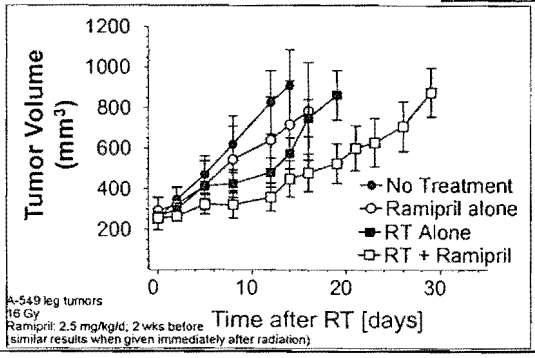



## ACE inhibitor: protect skin




## ACE inhibitor




## Other Promising Approaches

- Statins
- Pentoxifylline
- Vitamin E (tocopherol succinate)

## Conclusions

- Opportunities exist to use FDA approved (for other indications) pharmacological agents with anti-cancer intent that if timed properly will improve normal tissue response
  - HDACi on tissues: skin, oral mucosa, whole body
- Opportunities exist to use FDA approved (for other indications) pharmacological agents which reduce normal tissue injury and if timed properly will also exhibit anti-cancer activity
  - ACEi on multi-organ and tissues, e.g. lung, kidney, skin, CNS

## Unanswered Questions

- What is the optimum time to administer drug?
- How long to continue giving drugs?
- What are the side-effects in irradiated patients?
- Is mitigation organ specific?
- Is lack of effect on tumor, also true for "cancer stem cell"?

## Future Work

- ACEi: need for more preclinical work on tumor tissues
- HDACi: need for more preclinical work on normal tissue mitigation (other than skin, oral mucosa, whole body)
- Need to further study the mechanism of differential effects on tumor/normal tissue for both HDACi and ACEi as well as other compounds.

## Acknowledgements


- Jae Ho Kim, MD, PhD
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- Ben Movsas, MD

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(No summary received)

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Anthony J. Murgo, M.D.

Phase 0 Trials

A leading cause of failure of drugs in clinical development is lack of efficacy, due in good part to inadequate predictive animal models and poorly informed clinical trials. Failure rate may be reduced by putting more emphasis on establishing drug effects in the earliest phases of clinical development, eliminating "bad drugs" early and better informing subsequent trials of promising drugs. Phase 0 trials are designed primarily to evaluate the pharmacodynamic and/or pharmacokinetic properties of investigational agents in a relatively small number of patients before initiating larger traditional phase I studies.

One type of phase 0 trial is designed to evaluate the effect of a drug on its molecular target or pathway in human samples, utilizing and refining procedures developed and validated in preclinical models. Because of the limited number of patients and tissue samples, demonstration of target modulation in phase 0 trials requires a robust drug effect and a precise and reproducible assay. Since phase 0 trials involve extremely low doses administered over a short period, they may be initiated in accordance with the FDA Exploratory IND Guidance with less preclinical toxicity data than usually required for traditional first-in-human studies. Due to the very limited drug exposure and/or nature of the study agent, phase 0 trials offer no chance of therapeutic benefit. This presents ethical considerations and makes subject accrual challenging, particularly if invasive biopsies are involved. These difficulties may be overcome by tailoring study designs that are attentive to feasibility and risk minimization (e.g., use of surrogate tissues such as skin or peripheral blood cells). The first step in contemplating a phase 0 trial is selecting an appropriate study drug. The properties of an ideal drug candidate for a phase 0 trial to evaluate target/biomarker effect include: 1) a wide therapeutic window is expected; 2) target/biomarker modulation is anticipated and measurable post-treatment with low nontoxic doses given for short durations of exposure (e.g., $\leq$7 days); 3) an effect that can be adequately assessed in a small number of patients ($\approx$10 to 15) using analytical assay methods validated in preclinical models. These criteria apply to novel therapeutics, imaging probes, and biomodulators, including radiation injury mitigating agents. Well-designed and executed phase 0 trials are feasible and have potential for improving the efficiency and success of subsequent trials, particularly those evaluating molecularly targeted agents.

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## Phase 0 Trials

Role in Radiation Mitigation Agent Development?

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Jan 25, 2010



## Subject Matter

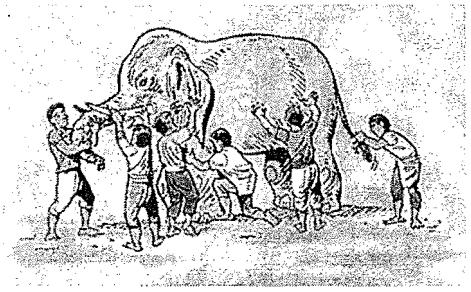
- Design and goals of Phase 0 (Exploratory IND) trials
- Phase 0 vs. traditional first-in-human Phase 1 trials
- What types of agents are candidates for Phase 0 testing



## Disclosure



## What is a Phase 0 trial?





## Basic Features of a Phase 0 trial

- First-in-human trial conducted prior to traditional Phase 1 study
- Small number of subjects (≈10-15)
- Limited drug exposure
  - Low, non-toxic doses
  - Short duration (≈ ≤7 days)
  - One course only
- No therapeutic intent
- Phase 0 trials are not definitive studies



## Phase 0 trials can start earlier than Phase 1

**Guidance for Industry, Investigators, and Reviewers**

**Exploratory IND Studies**

Conceived under FDA's "Critical Path" initiative to help sponsors identify promising candidate drugs more quickly

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

January 2006
Pharmacology/Toxicology

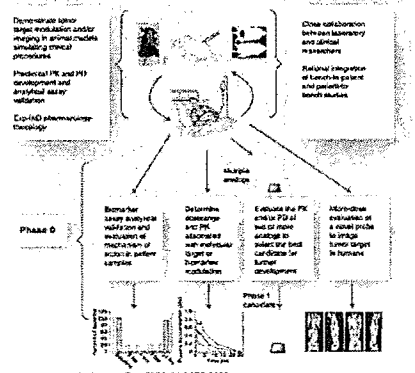
Toxicology evaluation less extensive than for traditional IND because of reduced dosing and limited exposure.

## Phase 0 vs. FIH Phase 1 Oncology Trials

| Variable | Phase 1 Trial | Phase 0 Trial |
|---|---|---|
| Preclinical tox | Full IND-directed | Less, sufficient to support ExpIND |
| Pre-clinical biomarker studies | Not consistently performed;assays rarely validated in preclinical models | Target/biomarker analytical assays validated in preclinical |
| Primary end-point | Establish dose-limiting toxicities and MTD or RPTD | Establish a dose-range that modulates target, for use in subsequent Phase 1 (or 2 trials) |

7

## Phase 0 Trial Designs




Murgo, A. J. et al. Clin Cancer Res 2008;14:3675-3682

10

## Phase 0 vs. FIH Phase 1 Oncology Trials (cont.)

| Variable | Phase 1 Trial | Phase 0 Trial |
|---|---|---|
| Duration of dosing | Repeated dosing with multiple cycles until disease progression or unacceptable toxicity | Limited dosing (e.g., 1-7 days); one cycle only |
| Evaluation for therapeutic benefit | Tumor response routinely evaluated | None |
| PD/target effect assays | Not consistently performed; commonly use assays that are not validated or standardized | Integrated into the trial to establish drug target effect; use and refine validated assay methods in patient tissue samples |

8

## Types of Phase 0/Expl IND trials

- Pharm acologically relevant doses
- Micro-dose studies

11

## Phase 0 vs. FIH Phase 1 Oncology Trials (cont.)

| Variable | Phase 1 Trial | Phase 0 Trial |
|---|---|---|
| Tumor Biopsies | Almost always optional | At least one pre- and one post-drug administration biopsy required to evaluate drug effect |
| SOP's for tissue acquisition, handling, and processing | Generally not validated or standardized | Reliable SOP's validated first in *in vivo* preclinical models and applied to Phase 0 human samples (export for Phase 1-2) |
| PK/PD analysis | Samples usually batched and analyzed at a later time point, generally after completion of the trial | Performed in real-time |

9

## Various Goals of Phase 0/Expl IND trials

### Pharmacologically relevant doses

- Explore mechanism of action in humans
  - MOA defined in non-clinical models can be observed in humans
  - Agent binds to or inhibits its alleged target
- Refine a biomarker assay using human tumor tissue and/or surrogate tissue
- Provide human PK-PD relationship data prior to definitive single-agent or combination Phase 1 testing
- Select most promising candidate for further development
  - Evaluate human PD of two or more analogs directed at same target and possessing practically the same preclinical properties

## Various Goals of Phase 0/Expl IND trials(2)

### Micro-dose studies

- Less than 1/100th of the dose calculated (based on animal data) to yield a pharmacologic effect (max dose of <100 micrograms (≤30 nanomoles, protein products)
- Evaluate in humans an agent's biodistribution, binding characteristics and target effects
- Develop novel imaging probes
- Evaluate human PK (e.g., bioavailability) to select most promising candidate for further development

13

## Phase 0 – Small Sample Size

- Demonstration target modulation requires:
  - Precise and reproducible assay methods
  - Robust drug effect
  - Limited intra-patient variability
  - Limited inter-patient variability
  - Innovative, rational statistical designs

14

## Phase 0 trials Can Improve Efficiency of Subsequent Trials

### Informing subsequent trials

- Refining PD analytical assay with human biopsy samples
- Developing reliable SOP for human tissue acquisition, handling, and processing
- Determining dose and time course that yields desired target effect
- Explore PK-PD relationships
  - closer approximation to a safe, efficacious starting dose
  - support for limited sampling in subsequent trials

15

## Phase 0 trials Can Improve Efficiency of Subsequent Trials (cont.)

- Selecting a candidate agent with most favorable properties for further clinical testing
- Eliminating "bad" agents early in clinical development because of poor PD or PK properties
  - e.g., lack of target effect, poor bioavail., very rapid clearance

"Fail fast, fail early"

16

## What makes an agent a good candidate for a phase 0 PD trial

- Credentialed target (modulation results in desired effect)
- Pre-clinical data show wide therapeutic window
- PD modulation expected at low doses and short duration of exposure (e.g. ≤7 days)
- Drug target effect can be established with a relatively small sample size (≤10-15 patients)
  - Requires robust effect and assay

17

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Phase 0 Clinical Trial of the Poly (ADP-Ribose) Polymerase Inhibitor ABT-888 in Patients With Advanced Malignancies

*Shivaani Kummar, Robert Kinders, Martin E. Gutierrez, Larry Rubinstein, Ralph E. Parchment, Lawrence R. Phillips, Jingping Ji, Anne Monks, Jennifer A. Low, Alice Chen, Anthony J. Murgo, Jerry Collins, Seth M. Steinberg, Helen Shih-peiho, Vincent L. Giranda, Gary Gordon, Lee Helman, Robert Wiltrout, Joseph E. Tomaszewski, and James H. Doroshow*

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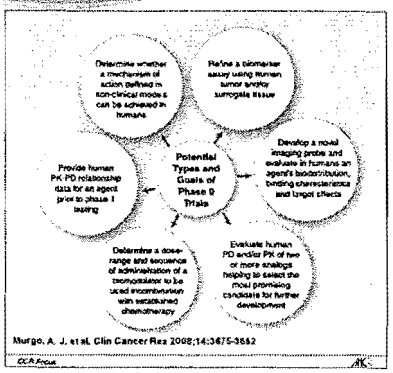



19

## Phase 0 – Patient Recruitment and Ethical Considerations

**Challenging, but not insurmountable**

- Potential barriers to patient enrollment
  - No therapeutic intent or chance of benefit - but low risk
  - Pre- and post-treatment tissue biopsies - avoid if possible
  - Delay or exclusion from other trials or therapies
    - washout period for phase 0 shorter

External concerns about ethics and availability of patients for study

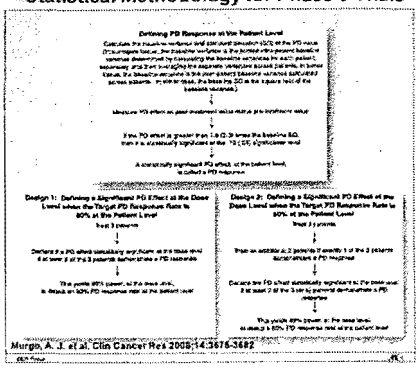
- Institutional Ethics committee review and input
- IRB approval
- Informed Consent Process
  - Clearly explain the rationale for the study
  - Clearly describe the limited treatment and follow up period
  - Clearly state that there is *absolutely no anticipated clinical benefit to the participant*
  - More straightforward than Phase 1

22

## Suggested Reading


- Kummar, S., et al. Phase 0 Clinical Trial of the Poly (ADP-Ribose) Polymerase Inhibitor ABT-888 in Patients With Advanced Malignancies. J Clin Oncol; 27:2705-11, 2009
- Kinders, R., et al. Phase 0 trials in cancer drug development. Mol Interventions; 7:327-34, 2007
- Jacobson-Kram D, Mills G. Leveraging exploratory investigational new drug studies to accelerate drug development. Clin Cancer Res;14:3670–4, 2008
- Murgo AJ, et al. Designing phase 0 cancer clinical trials. Clin Cancer Res; 14:3675-82, 2008
- Doroshow, JH, Parchment, RE. Oncologic Phase 0 Trials: Incorporating Clinical Pharmacodynamics from Concept to Patient. Clin Cancer Res 14:3658-63, 2008
- Gutierrez, M, Collyar, D. Patient perspectives on phase 0 clinical trials. Clin Cancer Res 14:3689–91, 2008
- Abdoler E, et. al. The ethics of phase 0 oncology trials. Clin Cancer Res 2008;14:3692–7
- Kummar, S, et al. Compressing drug development timelines in oncology using phase '0' trials. Nature Reviews Cancer 7:131-9, 2007
- Rubinstein, LV, et al. The Statistics of Phase 0 Trials. Statistics in Med; in press.


## Statistical Methodology for Phase 0 Trials




23

# Back-up Slides

21

# PANEL 5

**Benjamin Movsas, M.D., Chair, Radiation Oncology, Henry Ford Hospital**

Lessons Learned from RTOG 9801

RTOG 9801 was the largest randomized trial to test the ability of amifostine, a radioprotector, to reduce RT esophagitis in the setting of lung cancer. An early challenge related to slow accrual and one recurring issue was the concern among some clinicians re: potential tumor protection. This is despite the fact that many randomized clinical trials have not shown a significant difference in response rates, time to progression, or survival with amifostine. In *Lancet Oncology* (June 2003), there was a heated debate regarding this issue. Dr. Overgaard argued that "there are insufficient data to establish whether the use of amifostine decreases the rate of cure" and that the "absence of evidence is not evidence of absence". Yet, Dr. Brizel countered that "to absolutely refute claims that antitumor efficacy is compromised by amifostine, an equivalence trial would have to be done" which "would require >1200 patients per arm". Ultimately, RTOG 9801 met its accrual goal (N=243) and showed no difference in PFS or OS between the two arms. Nevertheless, in designing future clinical trials for RT mitigators, we need to be aware of this ongoing debate, particularly when studying relatively new agents.

Once a symptom management study is completed, the next challenge relates to how to correctly interpret the results. What endpoint/perspective matters most....that measured by the healthcare provider or reported by the patient? RTOG 9801 demonstrated the "disconnect" that can occur between these two perspectives. While the primary endpoint of the study (the maximum NCI-CTC esophagitis grade between arms) was negative (p=0.9), patients on the amifostine arm self-reported less swallowing symptoms on their daily swallowing diaries (p=0.025). Moreover, using a validated QOL instrument, patients on the amifostine arm reported significantly less deterioration in clinically meaningful pain scores (p=0.003). Thus, RTOG 9801, the only randomized study of amifostine in lung cancer to incorporate QOL, highlighted a fundamental disconnect between physician vs. patient reported outcomes (PROs). It is critical that this issue be addressed in designing clinical trials for mitigators of RT related toxicities.

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## Lessons from RTOG 9801

Benjamin Movsas, M.D.
Chairman, Radiation Oncology
Henry Ford Health System
Herndon Chair in Oncology Research

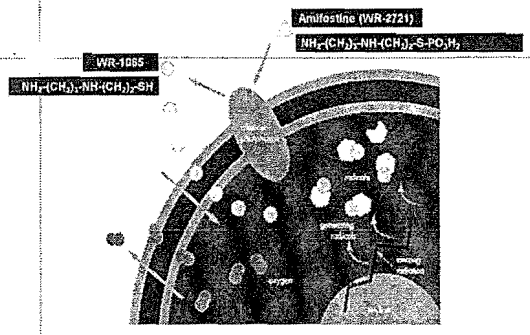
Lesson #1: Being PI of a study in not all fun and games!

## RTOG 9801

Induction P/C X 2 → BID RT + weekly P/C → Amifostine (500 mg IV) 4x/week (during chemoRT)

→ No Amifostine

## Amifostine: Mechanism of Action




Capizzi RL. *Oncology*. 1999;13:47-59

## RTOG 98-01

- Largest phase III trial of amifostine (n=243)
- In the setting of intensive chemoRT
- Collected prospective QOL data

## RTOG 98-01: Lesson #2

"The worst result of a clinical trial.....

## RTOG 98-01: Lesson #2

"The worst result of a clinical trial..... is no result at all!"

## RTOG 98-01

- Early on, the accrual was lower than projected
- While there were many issues (eg, activation issues, intensity of tx), one concern surfaced over time.....

## RTOG 98-01

- Early on, the accrual was lower than projected
- While there were many issues (eg, activation issues, intensity of tx), one concern surfaced over time.....
- POTENTIAL FOR TUMOR PROTECTION

## TUMOR PROTECTION?

- To date, there is no clinical evidence that amifostine protects tumors
- In many RCTs, a sig diff has not been seen in RRs, TTP, or OS

## TUMOR PROTECTION?

- Yet, this debate has a life of its own.....
- In *Lancet Oncology* (Vol 4, June 2003), there was a heated debate bwn Dr. Brizel and Dr. Overgaard

## TUMOR PROTECTION? Dr. Overgaard: YES

- "There are insufficient data to establish whether the use of AM decreases the rate of cure"
- "We should not forget that absence of evidence is not evidence of absence"

## TUMOR PROTECTION?
## Dr. Brizel: No

- In his RCT for H&N (N=303), 2 yr OS was 81% (AM arm) vs. 73% (no-AM)
- Odds ratio 1.12 (95% CI 0.98-1.27)
- "Critics argue that this trial was not sufficiently powered to detect a very small diff in survival. This argument is technically correct, but overlooks the realities of clinical trials and practice"

## TUMOR PROTECTION?
## Dr. Brizel: No

- "In order to absolutely refute the claims that antitumor efficacy is compromised by AM, an equivalence trial would have to be done.
- To show AM reduced survival from a hypothetical 45% to 40% (alpha=0.05, 80% power) would require >1200 pts per arm
- Yet, the largest H&N RCT took 8 yrs to accrue 1100 pts"

## TUMOR PROTECTION?
## Dr. Brizel: No

- "Tumor protection will always be a potential risk of any cytoprotective strategy, pharmacological or physical" (including, eg, IMRT)
- "Risks are inherent in the adoption of any new treatment paradigm. The greatest risk, however, is to simply ignore the tools available to us."

## Lesson #3:
## TUMOR PROTECTION

- In designing clinical trials for RT mitigators, we need to be aware of this ongoing debate, particularly as we embark on studying relatively new agents.

## RTOG 9801: Patient Accrual

| | |
|---|---|
| Total Patients Entered | 243 |
| Average Monthly Accrual | 5.7 |

## RTOG 9801:
## Survival and PFS (in months)

| | Amifostine | No-AM |
|---|---|---|
| Median Surv | 17.3 | 17.9 |
| Median PFS | 9.2 | 9.2 |

p = NS

## Lesson #4: The "disconnect"

- Once your symptom management study is completed.....how do you interpret the results?
- What endpoint/perspective matters most? That measured by the healthcare provider (MD) or reported by the patient (Patient Reported Outcome or PRO)?

## Two Methods of Assessing Outcome

- Maximum Esophagitis Grade (CTC).....measured by the MD (the "classic" primary endpoint)
- Patient Swallowing Questionnaire (patients were asked to assign a daily swallowing score 0-5 based on their symptoms; allows for Area Under The Curve calculation) + validated QOL instrument (EORTC QLQC30 + lung module)....ie, the PROs

## *Esophagitis Evaluation by MDs*

## Severe Acute Esophagitis

(Primary Endpoint)

| Amifostine (n = 120) Grade | | | No Amifostine (n = 122) Grade | | |
|---|---|---|---|---|---|
| 3 | 4 | 5 | 3 | 4 | 5 |
| 34 (28%) | 2 (2%) | 0 | 37 (31%) | 3 (3%) | 0 |

$p = 0.9$

Movsas *et. al. J. Clin. Oncol. 23:* 2145-54, 2005

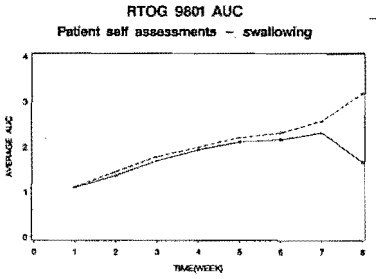
## *Esophagitis Evaluation by Patient Swallowing Log (2nd method)*

## Area Under the Curve During CT/RT At Least 15 Assessments Performed

| | | |
|---|---|---|
| **Range** | 1–3.76 | 1–3.5 |

**p = 0.025**

## Patient Self-Assessment–AUC
(solid line: amifostine)




**Lesson: Continue to collect PRO data over time!**

## QOL Endpoint

- In global assessment and subscales no significant differences between arms were seen.
- However, there was significantly less deterioration in clinically meaningful pain scores on the amifostine arm (compared to the other arm)--
  21% vs. 35% (p=0.003)

## Conclusions

- Amifostine did not reduce severe esophagitis in patients with lung cancer receiving concurrent chemotherapyand hyperfractionated RT.
- However, based on patient swallowing diaries, area under the curve of esophagitis was significantly lower with amifostine.

## RTOG 9801

**While the study did not show a decrease in the rate of severe esophagitis (using NCI-CTC criteria), patients who received amifostine self-reported significantly less swallowing symptoms (on pt diaries) and decreased pain (on their QOL forms).**

**RTOG 9801 highlighted a critical "disconnect" between physician vs. patient reported outcomes (PROs).**

Movsas *et. al. J. Clin. Oncol. 23:* 2145-54, 2005



## RTOG 9801

**Which begs a fundamental question:**

**Which of these perspectives is "right"?**



## RTOG 9801

**Which begs a fundamental question:**

**Which of these perspectives is "right"?**

**Some clinicians feel that PROs are "soft" measures that should be secondary to clinical or more "objective" endpoints.**

## METHODS

These pre-tx factors were analyzed on MVA as predictors for OS:

- -KPS (70-80 vs. 90-100)
- -Gender
- -Race
- -Histology (SqCCa vs. other)
- -Tx arm [AM vs. no-AM]
- -AJCC stage (II/IIIA vs. IIIB)
- -Age
- -Marital Status
- -Tumor location (lower vs. other)
- -Global QOL score (via validated EORTC-QLQ-C30)

Note: Only pts with <5% weight loss within 3 months were eligible for enrollment

AM = amifostine



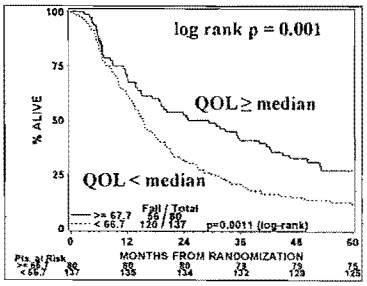
## RESULTS

When added to known prognostic factors, the baseline global QOL score replaced them all as the sole predictor of OS for patients with locally advanced NSCLC.

A clinically meaningful increase in the QOL score (of 10 points) corresponded to a decrease in the hazard of death by 10% (p=0.002)



## RESULTS




5 yr OS

For all pts: 17%*

27%

11%



## RTOG 9801

Which begs a fundamental question:

**Which of these perspectives is "right"?**



## RTOG 9801

Which begs a fundamental question:

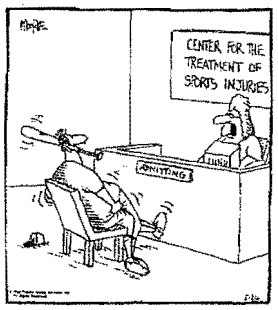
**Which of these perspectives is "right"?**

**BOTH!**



## STATISTICAL CONSIDERATIONS

- Missing data is a challenge that plagues most QOL studies.
- In RTOG 0214 (PCI NSCLC), only 50% of patients completed QOL at 6 months…and only 38% at 12 months.




"... Stop squirming, Mr. Silcox. The sooner we fill out these forms, the sooner we'll find out exactly what it is that's wrong with you."

**Across cultures, nobody likes a lot of paperwork!**

**Consider the burden!**



## STATISTICAL CONSIDERATIONS

**Missing Items**

- A strategy to check compliance with QOL timepoints using a real time electronic tracking system should be considered.
- Unlike traditional endpoints (like survival), QOL data cannot be collected retrospectively.

visionrec optimal care

RTOG
RADIATION THERAPY ONCOLOGY GROUP

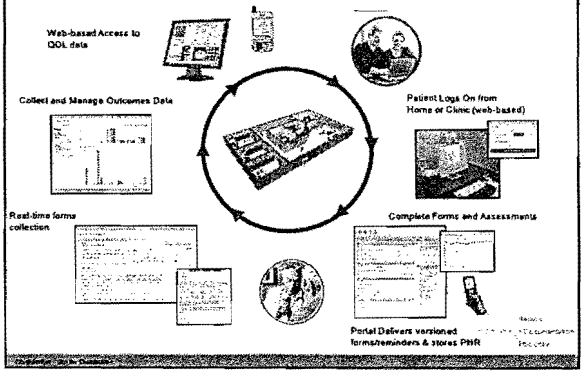
RTOG 0828 – A Pilot Companion Study To: 0415 A Phase III Randomized Study of Hypofractionated 3D-CRT/IMRT Versus Conventionally Fractionated 3D-CRT/IMRT in Patients With Favorable-Risk Prostate Cancer

## RTOG 0828 Study Background

RTOG 0828 Study Background

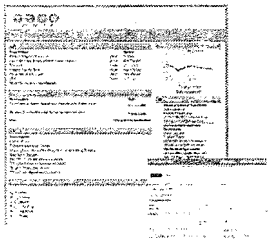
- Benjamin Movsas, PI
- Deb Bruner and Robert Lee, co-PIs
- RTOG 0828 pilot – a potential solution to help capture missing QOL data
  - *Challenges:*
    - *Cannot obtain QOL data retrospectively*
    - *Statistical analysis impacted*
- Web-based system being piloted to allow:
  - Consenting patients to complete QOL from any location
  - Remind patients (and RA's) if a QOL timepoint window is about to close before the data becomes 'missing'.
- Pilot Goal: To improve 6-month QOL data capture from ~50% to 80% (pilot study limited to interested 15 top accruing institutions) N ~ 100

## System Workflow




## Information Delivery Engine (IDE)

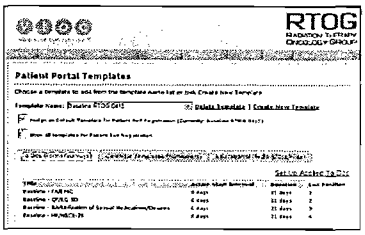
Patient's portal is auto-populated from RTOG 0828 study template



Clinical Trials Management – Deliver outcomes assessments during designated window and interval with related messages

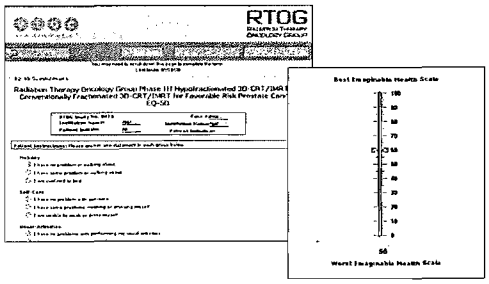
Patient Portal – clinical trials forms, consents, messaging and reminders. "Electronic Clipboard"

## Templatized Study Management




Study templates auto-deliver versioned forms, and reminders

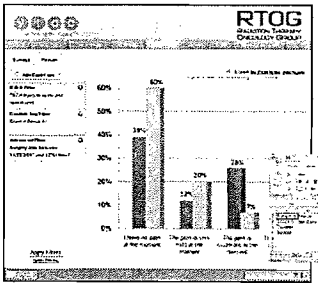
## QOL Study Forms for Online Completion




All study forms are provided to the patient for completion as they are on paper

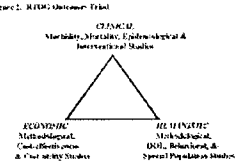
EQ5D_html.htm

## RTOG 0828 Reports and Data Filters




- Data entered by patients is De-identified and aggregated
- Custom reports may be generated
- Data exported to Excel, XML, PDF

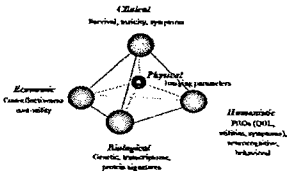
## CONCLUSION





## CONCLUSION

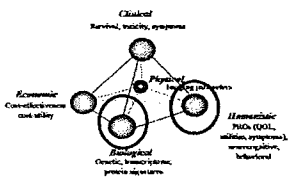
RTOG Outcomes Model (2007-2013)





## CONCLUSION

RTOG Outcomes Model (2007-2013)



## What are we learning about the QOL of cancer patients?

- We have known that it is related to:
  - recent life events (sun)
  - socioeconomic status (soil)
- Recent results suggest that we should now consider a role for genetics (the seed) in QOL.



## QOL and Genetics

The Establishment of the GENEQOL Disposition of Patient-Reported Consortium to Investigate the Genetic Quality-of-Life Outcomes

Mirjam A. G. Sprangers1, Jeff A. Sloan2, Ruut Veenhoven3, Charles S. Cleeland4, Michele Y. Halyard5, Amy P. Abernethy6, Frank Baas7, Andrea M. Barsevick8, Meike Bartels9, Dorret I. Boomsma9, Cynthia Chauhan10, Amylou C. Dueck11, Marlene H. Frost12, Per Hall13, Pål Klepstad14, Nicholas G. Martin15, Christine Miaskowski16, Miriam Mosing15, Benjamin Movsas17, Cornelis J. F. Van Noorden18, Donald L. Patrick19, Nancy L. Pedersen13, Mary E. Ropka20, Quiling Shi4, Gen Shinozaki21, Jasvinder A. Singh22, Ping Yang23, and Ailko H. Zwinderman24

Twin Research and Human Genetics 12, 301–311, 2009

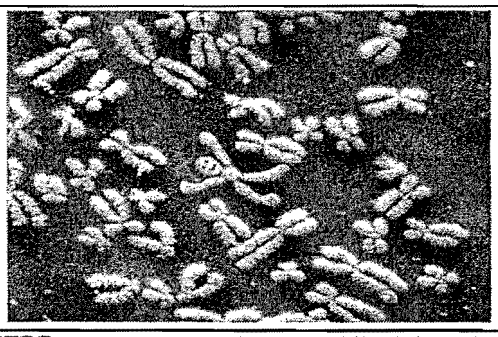


## Are you skeptical?.....GOOD!


"EUREKA!... I'VE DISCOVERED THE GENE THAT MAKES US THINK THAT EVERYTHING'S DETERMINED BY GENES!"



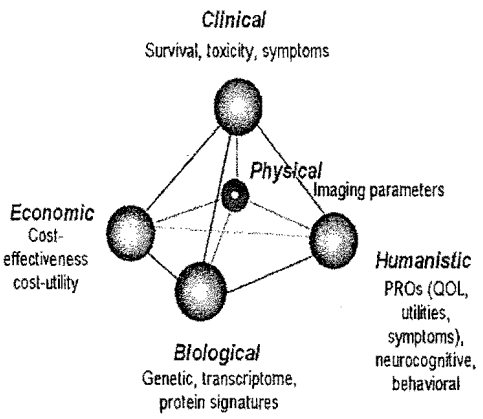
## Where is the QOL gene?



**Designing Phase II or III Clinical Trials to Demonstrate RT Mitigation: The RTOG Example**

Deborah Watkins Bruner, Ph.D., FAAN

The RTOG Health Services Research and Outcomes (HSRO) Committee has developed a framework to guide the assessment and testing of an increasingly comprehensive and complex set of outcomes in Phase II and III clinical treatment trials. The framework has evolved from a triad of clinical, humanistic and economic endpoints to a model that includes biological and physical outcomes. The goal is the collection of data that will help inform our understanding of the mechanisms and effects of RT on normal tissue and the impact on the patient symptom experience. The Figure below represents the latest version of this framework.




The success of the RTOG Outcomes Model in guiding the choice of endpoints in particular trials has led to wide acceptance in the group. However, not all RTOG trials are guided by this model because not all would benefit from this framework and because resources are finite. The reality of limited resources has directed strategies to prioritize the use of the model, including focusing on phase III trials and locating external funding for the biological endpoints.

The above Model is primarily used in treatment trials where resources are more extensive than in symptom management trials conducted through the RTOG Community Clinical Oncology Program (CCOP). However, the development of the NCI Symptom Management and Health Related QoL Steering Committee over the past 2 years has set the bar higher for the development of CCOP symptom management protocols with regard to the pre-clinical and pilot or phase I data required to move a concept forward. To meet this bar we have incorporated the framework set forth by the NCI Translational Research Working Group (TRWG). The TRWG conceptualizes translational research as a set of six developmental pathways focused on various clinical goals (Hawk, Matrisian et al. 2008). The pathway most pertinent to our work in symptom management is the life-style alterations pathway which can be used to guide interventions for cancer prevention; behavioral interventions to improve patient's adherence and response to cancer treatment; studies to ameliorate side effects of cancer treatments and studies to improve QoL (Hawk, Greenwood et al. 2008).

The challenge in the CCOP is that the NCI does not fund pre-clinical evaluation, leaving the research bases to rely mostly on the literature or pharmaceutical company data. We work to leverage CCOP-funded resources to accomplish some of our pathway-related goals.

In summary, RTOG is using the Outcomes Model and the TRWG pathways to guide our strategic approach to the evaluation of RT trials of curative intent and of the readiness of symptom management interventions and agents to move forward into phase II or III CCOP trials. These models are not meant to be prescriptive but are to be used as a guide. This presentation will provide examples of how RTOG utilizes these models in the design of clinical trials.

Deborah Watkins Bruner Email: wbruner@nursing.upenn.edu

# Designing Phase II or III Clinical Trials to Demonstrate RT Mitigation: The RTOG Example

Deborah Watkins Bruner, PhD, FAAN
Independence Professor of Nursing Education
Interim Associate Dean for Research, SON
Co-Program Leader, Cancer Control
University of Pennsylvania
Vice-Chair for Outcomes & CCOP PI, RTOG



## RTOG Roadmap for Assessing and Testing Interventions for RT Normal Tissue Toxicities

- The Health Services Research and Outcomes Committee (HSRO) assists with design of endpoints in Phase II-III treatment trials to assess toxicities and symptoms and their impact on patient quality of life
- The Community Clinical Oncology Program (CCOP) facilitates the development of Phase II-III symptom management trails to mitigate the effects of RT



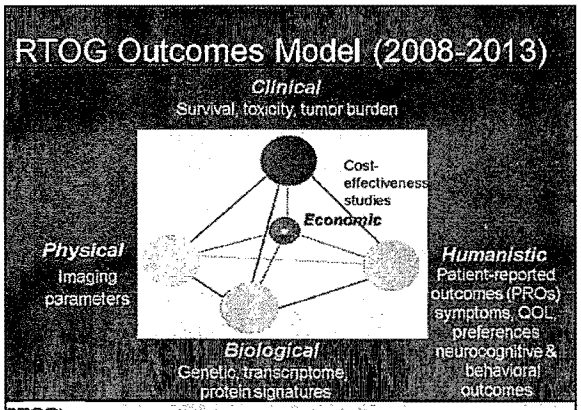
## Goals of HSRO

HSRO guides integration of traditional clinical outcomes with humanistic, economic, biological and physical outcomes to:

- Provide disease site and CCOP committees assistance with identifying protocols appropriate for the study of these individual and/or *integrated* endpoints.
- Assist with the identification of valid and reliable PRO and other measures and analyses.
  - In this capacity, the HSRO Committee service is analogous to a core facility, serving all RTOG CTEP and CCOP investigators and contributing to RTOG studies as needed.



## RTOG Outcomes Model (2008-2013)






Research Question: What are the interactions among clinical, humanistic and economic variables that optimize the outcomes and use of resources for defined populations?

RTOG 9714 Randomized Trial of Palliative RT for Osseous Mets from Breast or Prostate Ca Comparing 8Gy in 1Fx to 30Gy in 10Fxs

***Clinical***

455 pts in 8 Gy arm and 443 in 30 Gy arm. At 3 months:
- Grade 2–4 acute toxicity was more frequent with 30 Gy (17%) than with 8 Gy (10%, p<0.0001)
- BPI, CR and PR rates for 8 Gy were 15% and 50%, vs 18% and 48% for 30 Gy
- 33% no longer required narcotics

Hartsell, et al. *JNCI* 97: 798:2005

***Economic***

SUS/Quality-Adjusted Life Year (QALY) demonstrates the cost-benefit of the fraction schedules

Konski, et al, *AJCO* In Press

***Humanistic***

- No difference in overall RUI-III-HROL scores between arms at baseline or at 3 mos.
- Utility scores increased sig. from baseline to 3 mos.

Bruner, et al, *IJORBP* 60 (1) Supp:S142; 2003



RTOG 0534: Short Term Androgen Deprivation with Pelvic Lymph Node or Prostate Bed Only RT in Prostate Cancer Patients with a Rising PSA After Radical Prostatectomy (n=1764) (Phase III)

*RTOG Outcomes Model*

***Clinical***

Compare freedom from progression, toxicity, survival

***Physical***

Explore association between fatigue and energy delivered and volume of nml tissue irradiated

***Economic*** QALYs; cost-utility (modeled)

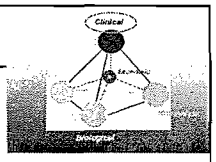
***Humanistic***

Compare QoL [EPIC, BFI, HSCL], Utilities [Eq5D]; Neurocognitive Fx

***Biological***

Explore predictive effects of markers on FFP, toxicity, QoL (e.g. fatigue & neurocogfx)

## Clinical

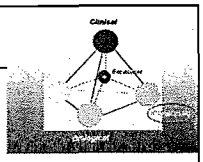


RTOG 0534: Prostate

- RT is the mainstay of salvage treatment for persistently detectable PSA or delayed rise
- There are no published salvage RT randomized trials
- *Primary objective:*
  - **Arm 1: PBRT Alone**
  - **Arm 2: PBRT + NC-STAD**
  - **Arm 3: PLNRT + PBRT + NC-STAD**
  - compare freedom from progression (FFP) for 5 years in men treated with salvage RT after radical prostatectomy

RTOG 7

## Humanistic

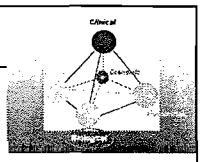


RTOG 0534: Prostate

| | Measures |
|---|---|
| • Side effects of pelvic RT: | |
| – Urinary | |
| – Bowel | EPIC |
| – Erectile dysfunction | |
| – Fatigue | BFI |
| • Side effects of AD: | |
| – Loss of libido | EPIC |
| – Erectile dysfunction | |
| – Hot flashes | |
| – Neurocognitivedysfunction | HVLT-R, Trail making A & B, COWAT |
| • In some pts PrCa associated with: | |
| – Depression | HSCL |

RTOG 8

## Biomarkers

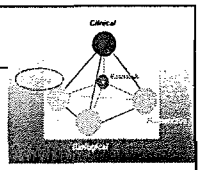


RTOG 0534: Prostate

- Clinical
  - PSA - DNA-ploidy - Ki-67
  - P53 - MDM2 - bcl-2
  - Bax - p16 - Cox-2
- Depression and Cognition
  - ADT associated with a significant rise in the plasma levels of the protein Abeta (associated w Alzheimer's) and with:
    - increased depression and anxiety scores
    - negatively correlated performance on word list memory test
  - Associations among testosterone, Abeta, depression and cognitive performance
- Fatigue
  - Pro-inflammatory cytokines IL1, IL6, TNF alpha, and IFN alpha associated fatigue
  - An exploratory aim is conduct genome-wide SNP analysis to identify candidate genetic pathways associated with fatigue

RTOG 9

## Physical

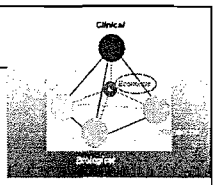


RTOG 0534: Prostate

- Fatigue has been found to increase significantly during the course of RT
- Etiology is not well understood
- The few reports that consider dose-volume related factors support the hypothesis that integrated dose (dose x volume) delivered may be a key factor
- We hypothesize that both dose intensity and variations in volume irradiated will be associated with fatigue (BPI) severity

RTOG 10

## Economic



RTOG 0534: Prostate

- Improvements in survival or progression-free survival comes at a cost – both financial and quality of life
- Calculation of the $/Quality Adjusted Life Year permits a summary equation allowing for differences in:
  - quality of life
  - clinical outcomes
  - cost to be incorporatedinto one equation
- This study will model cost-utility using the $U.S./QALY
  - We will model costs using Medicare reimbursementand measure utilities with the 5-item EQ-5D

RTOG 11

## CCOP Priorities

- Neurocognition
- Protectants for Mucositis
- Epithelial Injury
- Palliation & QoL
- Late Effects & Survivorship

RTOG 12

## http://www.cancer.gov/aboutnci/trwg/

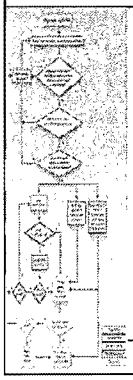
TRWG constructed 6 "developmental pathways" that characterize the transformation of scientific discoveries into new clinical modalities for oncology.

They fall into two complementary categories:

- Risk assessment modalities, intended to characterize the cancer-related health status of an individual:
  - Biospecimen-based risk assessment devices (protocols, reagents, instruments)
  - Image-based risk assessment (agents or techniques)
- Interventive modalities, intended to change the cancer-related health status of an individual, via prevention or treatment:
  - Agents (drugs or biologics)
  - Immune response modifiers (vaccines, cytokines, etc.)
  - Interventive devices
  - Lifestyle alterations

RTOG

## Common Structure

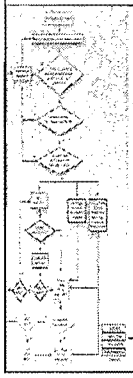


5 domains per pathway:

1) Credentialing - synthesis of literature to evaluate evidence of an underlying causal relationship between the discovery (e.g. agent or intervention) and outcome
2) Supporting tools - genomic, proteomic, metabolomic, imaging or other markers of risk or outcomes, or PROs that predict behaviors outcomes or are correlated with other markers
3) Creation of modality – development of an intervention
4) Preclinical development - animal models to provide data on biological or behavioral plausibility
5) Clinical trials – Phase I/II

*Hawk, Matrisian et al. Clin Cancer Res 2008;14(18)*

## The Radiation Therapy Oncology Group (RTOG) Community Clinical Oncology Program (CCOP)



The only CCOP research base focused solely on providing RT and RT combination therapy-related symptom amelioration trials to the community.

To judge the readiness of any agent or intervention for CCOP clinical trial development and to guide assessments of outcomes, we have adopted the NCI Translational Research Working Pathways model.

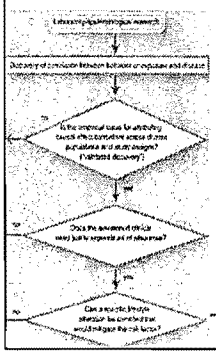
Once trials are judged ready for phase III design, we select a comprehensive set of outcomes guided by the RTOG Outcomes Model.

## Lifestyle Alterations Pathway

Memantine and Memory Training For Prevention of Cognitive Dysfunction in Patients Receiving Whole-brain Radiotherapy

RTOG

## Lifestyle Pathway: Credentialing: Scientific validation – RT Specific



- About 200,000 new cases in U.S. of primary or brain mets treated with RT annually
- Diffuse white matter injury, changes in glucose metabolism and neurocog changes occur at 40-50Gy *(Schultheiss et al 1995; Hahn et al 2008)*
- Hippocampal-dependent functions of learning, memory, and spatial information processing seem to be preferentially affected by RT *(Armstrong et al 2000; Monje et al 2002)*

## Lifestyle Pathway: Creation of Modality

Specify lifestyle alteration

*RTOG 0614 - open, (Paul Brown, PI)*

- WBRT 37.5Gy/15 fxs
  - Arm 1 + memantine - (Namenda™), an N-methyl-D-aspartate (NMDA) receptor antagonist, blocks inflammation
  - Arm 2 + placebo

Memory Training *(RTOG Concept in Development)* Exercises to:

- Enhance memory *(Morris 1996)*
- Hippocampal system intricately involved in function of memory; procedural memory can be established through brain mechanisms independent of the hippocampal system *(Nadel et al 2000)*.

RTOG

## Lifestyle Pathway: Supporting tools

Develop and validate biochemical, behavioral and/or imaging "assays" to measure effect of lifestyle alteration"

- RT specific tools
  - Dose X volume endpoint simulation trade-off curves –tx improvements compared to tx toxicities
    - Average change in MMSE score for those whose brain mets were radiologically controlled vs. those with uncontrolled brain mets
      - At 2 mos MMSE dropped average of 0.6 vs.1.9 for those with/without controlled brain mets ($p = 0.47$)
      - At 3 mos MMSE dropped 0.5 vs. 6.3 for those with/without controlled brain metastases ($p = 0.02$). *(Regine et al 2001)*
  - Recursive Partitioning
    - Class 1: patients with KPS > or = 70, < 65 years of age with controlled primary and no extracranial metastases
    - Class 3: KPS < 70
    - Class 2- all others *(Gaspar et al 1997)*
- Imaging techniques
  - Voxel based morphometry (VBM) and diffusion tensor imaging (DTI) MRIs *(Saykin)*
  - 2-deoxy-2-[18F]-fluoro-d-glucose (FDG)-PET (metabolism), 15O-PET (relative blood flow) *(Hahn et al 2008)*
  - fMRI - detect metabolic activation associated with cognitive processing , e.g. verbal declarative memory encoding task *(Freeman et al 2009)*

RTOG

19

## Lifestyle Pathway: Supporting tools

Develop and validate biochemical, behavioral and/or imaging "assays" to measure effect of lifestyle alteration"

- Genotyping arrays to assess genes associated with vulnerability *(Ahles et al 2007)* and those associated with successful treatment
  - In situ hybridization (ISH) documenteduregulation by memantineof 12 genes (e.g. GDP-dissociation inhibitor 1 (GDI-1), neural visinin Ca2+-binding protein 2 (NVP-2), neuromedin B receptor, and Na+/K+ transporting ATPase 2beta) in cortical and hippocampalregions *(Marvanova et al 2004)*
- Inflammatory microarrays
  - Inflammatory blockade can augment neurogensis after RT *(Monje et al 2003)*
  - Memantine
    - can protect against excitotoxicity from excessive exposure to glutamate which results in excessive Ca2+ influx through the receptor's associated ion channel
    - preferentially blocks excessive glutmate receptor activity without disrupting normal activity *(Lipton 2005)*

RTOG

20

## Lifestyle Pathway: Supporting tools

Develop and validate biochemical, behavioral and/or imaging "assays" to measure effect of lifestyle alteration"

- Neuropsychological assessments

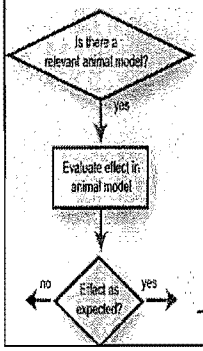
| | |
|---|---|
| | |
| | |
| | |
| | |
| | |

- Patient-reported outcomes
  - Functional Assessment Cancer Therapy- Brain

RTOG

21

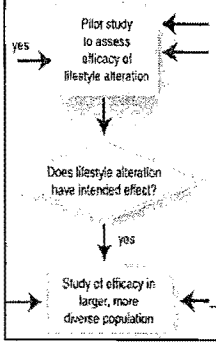
## Lifestyle Pathway: Preclinical Development




- Relevant mouse models have shown specific radiotherapy induced decrements in neurocog fx (Brown et al 2005, 2007; Monje 2003; Akayama et al 2001)
- Administration of inflammatory blockers can prevent or ameliorate radiation induced cognitive impairment in mouse models *(Monje et al 2003; Thotala et al 2008)*
- Environmental enrichment shown to promote neurogenesis and cognitive function after brain irradiation in gerbils *(Fan et al 2007)*

22

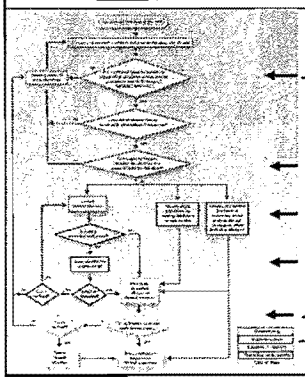
## Lifestyle Pathway: Clinical Trials




- Pilot study of patients undergoing radiotherapy to test feasibility and adherence to neurocognitive intervention
- Large scale multisite multimodal intervention in language and literacy diverse population

23

## Memantine and Neurocog Training After Brain RT



RT to brain causes white matter damage and particularly affects hippocampal-dependent functions of learning, memory, and spatial information processing

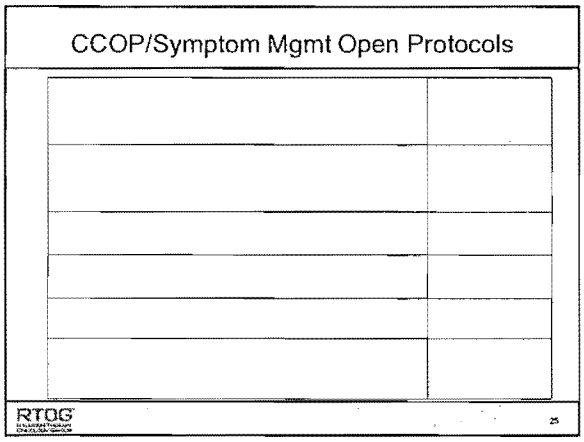
Medication plus memory training

Imaging, protein, inflammatory and genetic markers of neurocognitive function need further validation.

Animal models are relevant for assessing neurocognitive outcomes

Large scale multimodality study in primary or brain mets or PCI

24


CCOP/Symptom Mgmt Open Protocols
RTOG
25

# INDUSTRY REPS.

Contact: John Chant, PhD, chant_john@hotmail.com 650-307-7770

## G-Zero Therapeutics

**Norman (Ned) E. Sharpless MD. Founder**
Dr. Sharpless, an expert on the regulation of the cell cycle in cancer and aging, is Associate Professor of Medicine and Genetics at The University of North Carolina School of Medicine and an attending physician for hematological malignancies in the Lineberger Comprehensive Cancer Center at UNC. He is the author of over 60 publications and book chapters and is the inventor of two patents pending. Dr Sharpless' laboratory developed the intellectual property and methods that serve as the core technology for G-Zero Therapeutics. Dr. Sharpless received his clinical training in internal medicine and oncology at Harvard Medical School, The Massachusetts General Hospital, and The Dana-Farber Cancer Institute, where he also completed a Howard Hughes Medical Institute postdoctoral fellowship in the laboratory of Dr. Ronald DePinho. Dr. Sharpless holds an MD from UNC School of Medicine and BS in Mathematics from UNC where he was a Morehead Scholar. Dr. Sharpless has received several awards including an Ellison Medical Foundation New Scholar Award, a Paul Beeson Research Scholar Award, the Jefferson Pilot Award and elected membership into the American Society of Clinical Investigation. Dr. Sharpless is currently PI of three major grants from the NIH and Burroughs-Wellcome Foundation.

**Kwok-Kin Wong, MD, Ph D. Founder**
Dr Wong, currently Assistant Professor of Medicine at Dana Farber Cancer Institute and Harvard Medical School, studies lung cancer and signal transduction. He is an authority on EGFR signaling and genetically engineered mouse models of tumorigenesis. Dr Wong serves as an attending physician in oncology at DFCI, and he is the leader of several phase I trials. He has published over 40 major research papers. Dr Wong received his clinical training at DFCI, The Massachusetts General Hospital, and Brigham and Women's Hospital. At Harvard, Dr Wong worked as a HHMI Physician Scientist in the laboratory of Dr Ronald DePinho. Dr Wong received his MD/PhD from Columbia University, where he worked in the laboratory of Dr Katherine Calame, and BS in Biochemistry from Brown University. Dr Wong is the recipient of the Sidney Kimmel Cancer Research Scholar Award and elected membership into the American Society of Clinical Investigation. Dr Wong serves as PI on three major grants from the NIH.

**John Chant, Ph D. Founder, President, CEO**
Dr Chant currently serves as president and interim CEO for G-Zero. Currently, Dr Chant also works as a consultant to the Cancer Genome Project at Broad Institute and as a consultant to a large investor. Dr Chant served as Associate Director at Genentech, where he oversaw early stage receptor tyrosine kinase research and a major cancer genomics effort. While at Genentech, Dr Chant published four cancer genomics papers, authored three patents and led a venture capital investment in Complete Genomics, a next generation sequencing company. Prior to Genentech, Dr Chant worked for CuraGen/454 where he was Associate Director of Genomics, Proteomics, and Clinical Biomarkers. His accomplishments include 6 patent applications, two publications, and three alliances with pharmaceutical partners. Dr Chant was originally an academic on the faculty of Harvard University in Cambridge where he specialized in Genetics and Cell Biology. Dr Chant has received awards from the Searle Scholar Foundation, Damon Runyon Walter Winchell Cancer Research Fund, NIH, and American Cancer Society.

Contact: John Chant, PhD, chant_john@hotmail.com 650-307-7770

## Drug to Test: Oral CDK Inhibitors

- **First small molecule therapeutic for treating bone marrow suppression**
- **Therapeutic method prevents anemia, neutropenia, and thrombocytopenia**
- **Acute radiation sickness market: US Government supported**
- **Medical market: Oncology supportive care: augment or replace Epogen®, Neupogen®, and derivatives**
    - **$5B-plus markets**
- **Novel method: Strong intellectual property protection**
- **Novel mechanism (PharmacoQuiescence™): targets well-characterized (CDK: Cyclin-dependent kinases)**
- **Excellent data in mice – clinical evidence supports same mechanism in human**

G-Zero Therapeutics is a start-up with founders from the University of North Carolina School of Medicine and Harvard Medical School. G-Zero's founders have discovered a novel small molecule-based method for preventing the hematological side effects of radiation for treating acute radiation sickness. The same method also serves to protect bone marrow from the side effects of cancer chemotherapeutics. These are multibillion dollar markets. G-Zero's small molecule therapies act by a novel mechanism and have demonstrable advantages over existing therapies.

Bone marrow suppression is a severe consequence of exposure to radiation, and it is the cause of lethality following radiation exposure. Bone marrow suppression results in severe anemia, neutropenia, and thrombocytopenia (loss of red blood cells, white blood cells, and platelets). Existing therapies have major liabilities. First, they treat the symptoms rather than preventing bone marrow suppression. Second, as demonstrated within the past year, erythropoietin-based therapies (Amgen, JNJ) increase mortality in several patient classes. Third, no existing treatment prevents or treats loss of platelets. Finally, existing therapies are expensive-to-produce biologics that have short shelf life and can not be stockpiled effectively.

The G-Zero method for preventing bone marrow suppression overcomes these major liabilities of existing agents. Through use of orally available class of small molecules, the G-Zero method protects the bone marrow by inducing a block in cell division: "Pharmacological Quiescence™, PharmacoQuiescence™, or PQ™". To date, this method has proven highly effective in rodent studies, and strong clinical evidence exists that the same PQ™ mechanism exists in humans. It is also known that PQ™ agents have a favorable toxicity profile in humans. G-Zero is poised to perform confirmatory trials in larger animals and to commence human clinical trials.

Acute radiation sickness:: Given the global threat of radiological attack or disaster, the US government is committed to funding and stockpiling agents that protect against radiation sickness. Existing therapies that can be stockpiled (iodine, antibiotics) demonstrate only minor efficacy, while blood bank-based approaches (transfusion, stem cell transplants) are not practicable in a mass-casualty setting. Currently, there are no approved agents for "radiation mitigation"; that is, compounds that decrease toxicity when taken AFTER high-dose radiation exposure. In rodents, G-Zero's non-toxic and orally bioavailable small molecules demonstrate marked efficacy even when administered up to 20 hours following radiation exposure. Because acute radiation sickness would only occur under the circumstances of a calamity, conventional clinical trials in humans are not possible. Approval of therapies in the setting when human testing is not ethical is possible under the FDA's "Animal Rule." G-Zero is poised to meet the requirements of the Animal Rule to develop a first-in-class agent for radiation mitigation. Thus, acute radiation sickness represents a large

government-backed market where G-Zero can achieve rapid approval and revenue. We anticipate commercialization for the acute radiation sickness market in 24-36 months.

Cancer supportive care ($5-10B): The market for cancer supportive care to prevent bone marrow suppression exceeds $5B. Development for this market will require conventional clinical trials testing but the path to approval is conceptually straightforward. The drawbacks of existing therapies, as enumerated above, justify the development of G-Zero's agents. An orally available supportive therapy for bone marrow suppression would be the treatment of choice in chemotherapy and radiotherapy patients. Additionally, because PQ™ affords radioprotection through a novel mechanism that does not overlap with growth factor stimulation, this approach can be used as an adjunct to potentiate the efficacy of existing cytokine-based modalities. We anticipate commercialization in 36-48 months.

In summary, G-Zero has developed a novel and highly lucrative approach to preventing bone marrow suppression. G-Zero's simple and non-toxic approach reaches existing markets in the $5-10B range.

Contact: John Chant, PhD, President, CEO, chant_john@hotmail.com 650-307-7770

**Human mesenchymal stem cells overexpressing extracellular superoxide dismutase (ECSOD-hMSCs) for clinical trials as mitigation or therapeutics for cancer radiation therapy-induced normal tissue injury**

**Key personnel**

**Weiwen Deng, M.D., Ph.D., HCLD-Hematology (ABB)**
Spectrum Health,
Grand Rapids, Michigan

Dr. Deng, Research Director of Pediatric Blood & Bone Marrow Transplantation Program at Helen DeVos Children's Hospital, part of Spectrum Health, is a stem cell biologist with medical training background. He has been conducting "mesenchymal stem cells (MSCs) for gene and stem cell therapy" research over the past 11 years. Dr. Deng and his colleagues are the first to show that adenoviral-mediated MSC-based cell and gene therapy reverses erectile dysfunction and attenuates pulmonary hypertension in laboratory animals.

He and his colleagues have recently demonstrated for the first time that intravenous administration of MSCs overexpressing extracellular superoxide dismutase through adenoviral transduction (ECSOD-MSCs) improves survival, extends lifespan, retards cataract formation, and prevents carcinogenesis in irradiated mice (Abdel-Mageed et al., Blood 113: 1201-3, 2009; PCT/US09/48754 patent application entitled "Method for treating or preventing radiation damage using genetically modified mesenchymal stem cells", filed June 26, 2009). He is both the corresponding author of the paper and the principal inventor of the patent.

Dr. Deng predicts that systemic or local administration of ECSOD-MSCs could become a critical medical countermeasure against radiation. ECSOD-MSCs can be used as mitigation or therapeutics for radiation injury in nuclear/radiological emergency, space travel, and cancer radiation therapy.

He has an M.D. and an M.S. (Microbiology & Immunology) degree from Shanghai Medical University in China and a Ph.D. (Molecular & Cellular Biology) degree from Tulane University in USA. Dr. Deng holds the American Board of Bioanalysis (ABB) certification of High-complexity Clinical Laboratory Director (HCLD) in Hematology.

**Drug to propose for study in cancer patients**
**Human mesenchymal stem cells overexpressing extracellular superoxide dismutase (ECSOD-hMSCs)**

Radiation-induced normal tissue injury is the dose limiting factor in radiation therapy for cancer. The optimal cancer radiation therapy is to deliver radiation at a dose high enough to destroy cancer cells without exceeding the level that the surrounding healthy cells can tolerate. Currently, there is no approved mitigation or therapeutics available for normal tissue injury caused by cancer radiation therapy, which not only limits radiation dose escalation but also affects patient's quality of life. Therefore, the purpose of our proposed clinical trials is to develop mitigation or therapeutics for cancer radiation therapy-induced injury to normal cells in cancer vicinity, often leading to the failure of conventional radiation therapy.

Formation of superoxide anion ($O_2^-$) after ionizing radiation is a major determinant of radiation injury. Extracellular superoxide dismutase (ECSOD) is a potent antioxidant enzyme. Mesenchymal stem cells (MSCs), a subset of adult stem cells from bone marrow, migrate to radiation injured tissues after intravenous administration. To test our hypothesis that MSCs overexpressing ECSOD (ECSOD-MSCs) have a therapeutic effect for radiation injury, human and mouse MSCs (hMSCS and mMSCs) were transduced with Ad5CMVECSOD, an adenovirus carrying human ECSOD gene, and secretions of high level biologically active ECSOD were detected. The results of preliminary experiments in our laboratory show for the first time that intravenous administration of mouse MSCs overexpressing ECSOD (ECSOD-mMSCs) improved survival, extended lifespan, retarded cataract formation, and prevented carcinogenesis in irradiated mice (Abdel-Mageed et al., Blood 2009; 113:1201; Patent "PCT/US09/48754", filed June 26, 2009). Therefore, ECSOD-MSCs could become a critical medical countermeasure against radiation.

Here we propose to use our patented "ECSOD-MSCs for radiation injury" approach as mitigation or therapeutics for cancer radiation therapy-induced normal tissue injury to collaborate with clinicians working with cancer patients for clinical trials.

We will collect 10 ml bone marrow from a cancer patient for the isolation of hMSCs. We will ex vivo expand and gene engineer hMSCs with Ad5CMVECSOD so that the cells can secrete ECSOD in a cGMP laboratory. These ECSOD-hMSCs will be returned to the same patient through systemic or local administration prior to, during, or after cancer radiation therapy for mitigation or therapeutics of cancer radiation therapy-induced normal tissue injury. For example, we propose to conduct clinical trials investigating whether ECSOD-hMSCs can mitigate or treat radiation pneumonitis/fibrosis in breast cancer patients undergoing radiation therapy. If successful, ECSOD-hMSCs could be used as a high dose radiation therapy adjunct agent for the treatment of many types of cancers.

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## Synthetic SOD/catalase mimetics for mitigating radiation injury to normal tissues

### I. Key Personnel

**Boston University School of Medicine (BUSM),** Boston, MA:

Susan R. Doctrow, Ph.D. is the director of a Scientific Core, at BUSM, that is part of the Center for Medical Countermeasures Against Radiation (CMCR) based at Medical College of Wisconsin (MCW). She directly oversees all in-house research and works with the various contractors and consultants to coordinate the services to be provided by the Core, including development and analysis of SOD/catalase mimetics as mitigators of radiation injury. Dr. Doctrow has a Ph.D. in biochemistry (Brandeis University) and about 24 years' post-graduate experience in academic and biotechnology research, including the discovery and development of synthetic SOD/catalase mimetics. She was previously Vice President, Therapeutics Research at Proteome Systems, Inc., where she established this Scientific Core and directed it during previous years of the CMCR research. Prior to that, she was Vice President, Research at Eukarion, Inc. where she was an inventor of the synthetic SOD/catalase mimetics discussed in this summary. She continued to direct the Core after its move to the BUSM campus, where she is now Research Associate Professor of Medicine.

Rosalind Rosenthal, Ph.D. is a Research Associate in Dr. Doctrow's laboratory at BUSM. She has a Ph.D. in biochemistry (Brandeis University), previous postdoctoral experience in biochemistry and cell biology, and about 5 years' experience in biotechnology research. She has worked in the Scientific Core since its inception, originally as a Scientist at Protoeome Systems, and was the principal investigator on the Pilot grant that developed some new orally available EUK-400 compounds and demonstrated their mitigating efficacy in endothelial cell cultures. This research, conducted in collaboration with Dr. Susan Braunhut's laboratory at University of Massachusetts, Lowell, resulted in two publications on orally bioavailable SOD/catalase mimetics, as cited in the summary of those compounds.

Julie A. Straub, Ph.D. is working with Dr. Doctrow's lab as a consultant in drug development. She has a Ph.D. in Chemistry (MIT) and over 20 years experience in biotechnology, including research and development in areas of particular relevance to the CMCRs needs, including novel drug formulation and delivery systems and development of agents that modulate the vascular endothelium. Dr. Straub's experience ranges from supervising in-house scientific staff, to selecting, evaluating and overseeing contract research in key regulatory activities such as GLP pharmacology and toxicology and cGMP manufacture. In addition, she has expertise in developing novel therapeutic and diagnostics agents from the IND to the NDA level.

**Medical College of Wisconsin (MCW)**, Milwaukee, WI:

Zelmira Lazarova, M.D. is Assistant Professor of Dermatology at MCW, with 15 years post-graduate experience in cutaneous research. Dr. Lazarova is a dermatologist (MD, Comenius University, Slovakia), with prior experience at the NCI (Visiting Fellow) and Johns Hopkins University (Research Assistant Professor). As PI of a Pilot Grant funded by the MCW CMCR, Dr. Lazarova developed and characterized, in collaboration with Dr. John Moulder's laboratory, a rat model for cutaneous combined injury, involving both radiation and trauma, as described in this summary.

John E. Moulder, Ph.D. is Professor of Radiation Oncology, Radiology and Pharmacology at MCW and is the Director of the MCW CMCR. Dr. Moulder has a Ph.D. in Cell Biology (Yale University) and over 35 years experience in radiation biology, including pioneering work in the study of mitigators of radiation injury to normal tissues, in particular, the mitigation of renal injury by modulators of the renin-angiotensin system.

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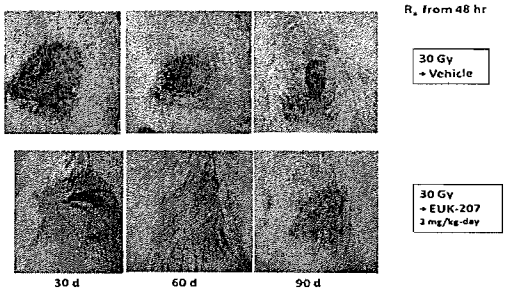
## II. EUK 207 and EUK-189: synthetic SOD/catalase mimetics to mitigate radiation injury to normal tissues, including the skin, lung, kidney, and CNS.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been implicated in chronic radiation-induced damage [1]. Chronic injury to tissues (*e.g.,* kidney, lung, skin, and brain) includes fibrosis, necrosis, atrophy, and vascular damage, occurring months to years after irradiation. Proinflammatory events and mitochondrial dysfunction have been implicated in chronic radiation injury, suggesting that agents to interrupt these damaging subcellular processes might have considerable therapeutic benefit against both acute and chronic radiation injury. Salen Mn complexes are synthetic low molecular weight agents that mimic the antioxidant enzymes superoxide dismutase (SOD) and catalase, scavenging superoxide and hydrogen peroxide/RNS, respectively [2]. Prototype salen Mn complexes are effective in a wide range of models for diseases involving oxidative stress [3]. Both EUK-189 and a newer cyclized analog EUK-207 improved function and suppressed brain oxidative stress in a mouse model for age-associated cognitive impairment [4, 5]. In various *in vivo* models for injury and degeneration, salen Mn complexes suppress proinflammatory processes as well as ROS- and RNS-associated macromolecular modifications [6-8]. They are also "mitoprotective", prolonging survival up to 3-fold, protecting mitochondrial enzymes, and preventing oxidative pathologies in a mouse model for mitochondrial oxidative stress [9, 10]. EUK-189 and EUK-207 were much more effective in this model than other agents tested (the SOD/catalase mimetic MnTBAP, the SOD mimetic M40403, mitochondrial metabolites, alpha lipoic acid and L-acetyl-carnitine) [3]. Such data led to their study as radiation mitigators in the CMCR program.

A single s.c. injection of EUK-189, at various times before or after irradiation, prevented lung micronucleus formation both in and out of field in the rat [11] and prolonged survival of mice after lethal irradiation [12]. Given s.c. or with a topical protocol, it also mitigated radiation-induced mucositis in a hamster model (unpublished findings), based on a clinically relevant severity score that has been described [13]. In the MCW CMCR, EUK-189 was tested in a rat renal injury model involving total body irradiation and bone marrow transplant (TBI/BMT) [14] but was not significantly effective. This led to evaluation of cyclized salen Mn complex, EUK-207, which has equivalent SOD and catalase activities, but greater stability [3, 4] and longer *in vivo* half-life in rats, given iv or sc. Given by continuous s.c. infusion (~8 mg/kg-day) for 12 wks, beginning 3 wks after TBI (9 Gy), EUK-207 mitigated renal injury while EUK-189 was again less effective [15]. Based on such findings, EUK-207 is a lead SOD/catalase mimetic for the MCW CMCR. It has since shown beneficial effects in lung and CNS radiation injury models (not shown), as well as in a combined cutaneous injury model (radiation to the skin plus full-thickness wounding) [16]. Rats received EUK-207 (~2 mg/kg-day) by continuous sc infusion, beginning 48 hr after radiation and wounding. The EUK-207 treated rats showed lower skin injury scores and faster and complete wound healing, compared to the vehicle treated rats [16]. (**Fig 1**). Vascular density in the wound area was markedly increased with EUK-207 (**Fig 2**), suggesting either protection of endothelium and/or stimulation of angiogenesis. By comparison, rats on a diet of 5% curcumin showed lower skin injury scores, but no change in wound healing. A topical formulation of EUK-189 was developed with prior funding from the NCI, and is also suitable for EUK-207. Thus, future plans will include study of both systemic and topical preparations for mitigation efficacy in the skin. Systemic studies in other target organs will continue.

**Fig1**



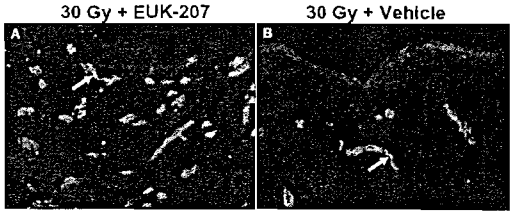




**Fig 2:** Staining with anti-CD31 specific antibody (blood vessels marker) showed statistically significant increase in the number of blood vessels in the EUK-207 treated group (A) compared to vehicle treated animals (B) [16].

## III. Orally-available Mn porphyrin SOD/catalase mimetics as potential mitigators of normal tissue injury

The salen Mn complexes discussed above are not orally bioavailable. They are given by injection, sc pump infusion, or, for certain indications, topically. Orally-bioavailable agents would be very convenient as radiation countermeasures, assuming GI injury does not hinder their ingestion. Pilot CMCR Research was conducted to investigate an orally-bioavailable class of Mn porphyrins, known as "EUK-400 compounds" (**Fig 3**) as radiation mitigators [17, 18]. Both the salen Mn complexes and EUK-400 compounds are anti-apoptotic in cell culture models [17], including mitigating radiation-induced apoptosis in microvascular endothelial cell cultures (**Fig 4**) [18]. We previously showed [17] that several EUK-400 compounds are orally available when given by intragastric gavage to rats. In our CMCR studies, we showed that EUK-451

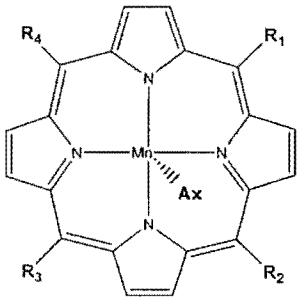



**Fig 3.** EUK-400s: R1,3= 4-tetrahydropyrano (EUK-451), phenyl (EUK-423) or cyclopropyl (EUK-418); R2,4=H; other structures in ref. 17.

is also bioavailable via drinking water. EUK-451, and potential back-up analogs, will be tested for mitigation of radiation injury *in vivo*. We have hypothesized that the two classes of compounds might act via different mechanisms or sites of action [17], so may serve as complementary agents for mitigating radiation injury.

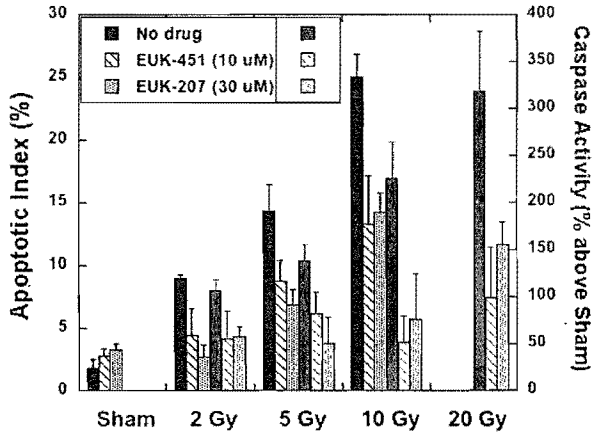



**Fig 4.** Bovine capillary endothelial cells were exposed to radiation (X-ray) at the indicated doses and compounds were given 1 hr after, and apoptosis assessed at 6 hr. Drug effects were significant in all groups except Sham-irradiated [18].


1. Robbins, M.E.C. and W. Zhao, In J Radiat Biol, 2004. **80**: p. 251-259.
2. Doctrow, S.R., et al., J Med Chem, 2002. **45**(20): p. 4549-58.
3. Doctrow, S.R., et al., in *Medicinal Inorganic Chemistry*, J. L Sessler, SR Doctrow, T. J. McMurry, and S. J. Lippard, Eds. 2005, ACS and Oxford University Press: New York. p. 319-347.
4. Liu, R., et al., Proc Natl Acad Sci U S A, 2003. **100**(14): p. 8526-31.
5. Clausen, A., S. Doctrow, and M. Baudry, Neurobiol Aging, online 2008.
6. Rong, Y., et al., Proc Natl Acad Sci U S A, 1999. **96**(17): p. 9897-902.
7. Zhang, H.J., et al., Faseb J, 2004. **18**(13): p. 1547-9.
8. Peng, J., et al., J Biol Chem, 2005. **280**(32): p. 29194-8.
9. Melov, S., et al., J Neurosci, 2001. **21**: p. 8348-8353.
10. Hinerfeld, D., et al., J. Neurochem., 2004. **88**: p. 657-667.
11. Langan, A.R., et al., Radiother Oncol, 2006. **79**(2): p. 231-8.
12. Srinivasan, V., et al., Immunopharmacol Immunotoxicol, 2008. **30**(2): p. 271-90.
13. Murphy, C.K., et al., Clin Cancer Res, 2008. **14**(13): p. 4292-7.
14. Moulder, J.E.et al., Int J Radiat Oncol Biol Phys, 1993. **27**(1): p. 93-9.
15. Doctrow, S.R., et al., Rad. Res. 55th Annual Meeting, 2009. **Abstract S1403**: p. 26.
16. Jourdan, M.M., et al., Rad. Res. 55th Annual Meeting, 2009. **Abstract PS1.35**: p. 64.
17. Rosenthal, R.A., et al., J Biol Inorg Chem, 2009. **14**(6): p. 979-91.
18. Vorotnikova, E., et al., in review.

**ORE1001/ORE PHARMACEUTICALS INC.**

15 December 2009

Ore overview and key personnel

Ore Pharmaceuticals Inc. has been a leader in the field of drug repositioning and is currently developing drugs based on the drug repositioning findings. The company has applied an integrative pharmacology approach to identify potential new uses for drug candidates that have failed clinical development for reasons other than safety.

Stephen Donahue, M.D. is the Senior Vice President of Clinical Development for Ore. His background includes management of drug development programs, all phases of clinical drug development and regulatory affairs. Prior to joining Ore, from 2004 to 2007, Stephen was Vice President, Clinical Research and Regulatory Affairs at Predix Pharmaceuticals. Prior to Predix, Stephen was at Merck & Co., Inc. in the department of Clinical Research. He started his drug development career at Bristol-Myers Squibb, where he held positions of increasing responsibility in the departments of Clinical Design/Evaluation and Clinical Pharmacology. He has worked on a number of approved drugs, including pravastatin, metformin, ezetimibe and sitagliptin. Stephen holds an AB from Brown University, an MD from Georgetown University, and completed a National Research Service Award Fellowship in Clinical Pharmacology at Georgetown. He has achieved board certifications in both Internal Medicine and Clinical Pharmacology.

John Reinhard PhD is currently directing the ORE1001 preclinical program as well as the clinical program for ORE1001 in ulcerative colitis. Dr. Reinhard received his PhD in biology from MIT and did postdoctoral training in Pharmacology at Yale, under the supervision of Dr Robert Roth. Dr Reinhard's professional career involved positions of increasing responsibility at Burroughs-Wellcome, Glaxo-Wellcome and GSK, initially in the non-clinical area and later in clinical development at Glaxo where he was involved in the development of of Zyban, Lamictal and Ziagen. Before joining Ore, Dr Reinhard was the Senior Director of Clinical Research at Epix Pharmaceuticals.

ORE1001

ORE1001 is a clinical stage, first-in-class, orally administered small molecule drug that is currently being tested in clinical trials as a potential treatment for Inflammatory Bowel Disease (IBD). ORE1001 is a potent inhibitor of the ACE2 enzyme. Animal studies show that ORE1001 reduces signs of injury and inflammation in experimental colitis, gastritis, gastric ulcer, and radiation-induced proctitis. An IND was filed June 30, 2008 and this has cleared review by the FDA. ORE1001 was investigated in a U.S. based multiple rising dose clinical study of 14 days dosing initiated in late 2008. It has completed testing in a clinical Phase I single-ascending dose study in the U.K. It was well-tolerated up to the highest dose tested in both the single and multiple dose studies, which is consistent with results of animal toxicity and safety studies. ORE1001 has a pharmacokinetic profile consistent with once or twice daily dosing. Ore is initiating a six week phase 1b/2a clinical trial in patients with IBD.

Data from disease expression databases revealed a linkage between ACE2 and inflammatory diseases of the gastrointestinal tract. In mice, NFkB pathway activation was reduced by ORE1001 treatment. In the mouse dextran sodium sulfate (DSS) model, ORE1001 dose-dependently reduced signs of disease activity, reduced histopathology scores, maintained colon length, and reduced tissue myeloperoxidase activity (Inflamm Res. 2009;58(11):819-27). In a rat model of gastritis induced by non-steroidal anti inflammatory drugs (NSAIDs), ORE1001 produced significant dose-dependent reduction in the severity of indomethacin-induced gastric damage. A second model with diclofenac further demonstrated that ORE1001 reduced gastric damage scores and myeloperoxidase activity. Oral administration of ORE1001 also improved disease measures relative to vehicle control in a rat chronic ulcer healing model. These data were further supported by a murine model of colitis induced by 7 days exposure to DSS in the drinking water. The severe colitis was associated with a 60% mortality in animals receiving vehicle. Co-administration of ORE1001 abolished the mortality caused by DSS.

The potential for ORE1001 as a radiation injury mitigation agent comes predominantly from a model of radiation-induced proctitis. In rats, exposure of the distal colon to 17.5 Gy of radiation resulted in significant endoscopic measures of pathology 8 days post-irradiation. Daily oral administration of ORE1001 significantly attenuated the endoscopic measures of damage. In a separate study, ORE1001 was administered to mice exposed to 13.5 Gy of whole-body irradiation. In contrast to the focal radiation study in rats, ORE1001 had no apparent effect on small intestine crypt survival when examined 4 days-post radiation.

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# Protective Role of R-spondin1, an Intestinal Stem Cell Growth Factor, against Radiation-Induced Gastrointestinal Syndrome in Mice

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## Abstract

*Background:* Radiation-induced gastrointestinal syndrome (RIGS) results from a combination of direct cytocidal effects on intestinal crypt and endothelial cells and subsequent loss of the mucosal barrier, resulting in electrolyte imbalance, diarrhea, weight loss, infection and mortality. Because R-spondin1 (Rspo1) acts as a mitogenic factor for intestinal stem cells, we hypothesized that systemic administration of Rspo1 would amplify the intestinal crypt cells and accelerate the regeneration of the irradiated intestine, thereby, ameliorating RIGS.

*Methods and Findings:* Male C57Bl/6 mice received recombinant adenovirus expressing human R-spondin1 (AdRspo1) or E.coli Lacz (AdLacz), 1–3 days before whole body irradiation (WBI) or abdominal irradiation (AIR). Post-irradiation survival was assessed by Kaplan Meier analysis. RIGS was assessed by histological examination of intestine after hematoxilin and eosin staining, immunohistochemical staining of BrdU incorporation, Lgr5 and β-catenin expression and TUNEL staining. The xylose absorption test (XAT) was performed to evaluate the functional integrity of the intestinal mucosal barrier. In order to examine the effect of R-spondin1 on tumor growth, AdRspo1 and AdLacZ was administered in the animals having palpable tumor and then exposed to AIR. There was a significant increase in survival in AdRspo1 cohorts compared to AdLacZ ($p<0.003$) controls, following WBI (10.4 Gy). Significant delay in tumor growth was observed after AIR in both cohorts AdRspo1 and AdLacZ but AdRspo1 treated animals showed improved survival compared to AdLacZ. Histological analysis and XAT demonstrated significant structural and functional regeneration of the intestine in irradiated animals following AdRspo1 treatment. Immunohistochemical analysis demonstrated an increase in Lgr5+ve crypt cells and the translocation of β-catenin from the cytosol to nucleus and upregulation of β-catenin target genes in AdRspo1-treated mice, as compared to AdLacz-treated mice.

*Conclusion:* Rspo1 promoted radioprotection against RIGS and improved survival of mice exposed to WBI. The mechanism was likely related to induction of the Wnt-β-catenin pathway and promotion of intestinal stem cell regeneration. Rspo1 has protective effect only on normal intestinal tissue but not in tumors after AIR and thereby may increase the therapeutic ratio of chemoradiation therapy in patients undergoing abdominal irradiation for GI malignancies.

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## Introduction

Normal homeostasis of intestinal epithelium is maintained by an intricate cell replacement process in which terminally differentiated epithelial cells are continuously and rapidly replaced by replication and differentiation of epithelial cells (transit cells) located within the intestinal crypts. Radiation-induced gastrointestinal syndrome (RIGS) is due in part to the killing of clonogenic crypt cells with eventual depopulation of the intestinal villi [1,2]. Crypt epithelial cells proliferate rapidly and are highly sensitive to cytotoxic agents and irradiation. Loss of this regenerating population of clonogenic cells following irradiation prevents the normal reepithelialization of the intestinal villi. This impairment leads to varying degrees of villous blunting and fusion, with attenuation and hypertrophy of the villous epithelial cells [3]. These changes result in the acute RIGS presenting with malabsorption, electrolyte imbalance, diarrhea, weight loss and potentially death. The late side effects and the sequelae of severe acute intestinal radiation injury include varying degrees of intestinal inflammation, mucosal thickening, collagen deposition, and fibrosis, as well as impairment of mucosal and motor functions [4,5,6]

The putative multipotent, intestinal stem cell is thought to be located at the base of the crypt, either at fourth or fifth cell position

from the base [7] or as crypt base columnar cells interspersed between Paneth cells [8]. In the normal state, these cells rarely proliferate unless there is a pressure for increased production of the clonogenic self-renewing progenitor cells, which undergo rapid clonal expansion, followed by differentiation into the mature cells lining the villi. The daughter cells migrate either toward the villus differentiating into enterocytes, goblet cells, and enteroendocrine cells, that are eventually shed into the gut, or inwards to the crypt bases giving rise to Paneth cells [9]. Thus, the multipotent cells are fundamental to the maintenance of the cell population of the intestinal epithelium and it's regeneration after injury [10]. Following exposure to ionizing radiation, cells located at the base of the crypt undergo rapid apoptosis, or stop dividing temporarily or permanently. The extent of cell loss and intestinal injury is dependent on the radiation dose [11]. Therefore, the fate of the crypt after injury is determined by replacement of the clonogenic proliferating crypt cells by intestinal stem cell. If all crypt cells die, the crypt is "sterilized" and disappears within 48 hours. However, if one or more 'clonogenic cell' survives the insult, it rapidly proliferates regenerating the crypt within 72–96 hours with subsequent reconstitutions of the villi. Survival of the animal depends on the balance between crypt depopulation, and the efficiency and number of the surviving clonogenic cells regenerating the crypts.

The β-catenin/T cell factor (TCF) signal transduction pathway plays a critical role in the regulation of proliferation and differentiation of the intestinal epithelial cells during the regeneration and maturation process along the crypt-villus axis [12,13]. Wnt signaling and the activation of β-catenin are important in the proliferation of the pluripotent stem cell that gives rise to crypt epithelial progenitors. The amount of Wnt proteins within the intestinal epithelial cells decreases with progression up the villus. As Wnt signaling decreases, β-catenin forms a complex with APC and axin (destruction complex), leading to the degradation of β-catenin [14]. Thus Wnt signaling is likely important to the maintenance of the undifferentiated state of intestinal crypt progenitor cells [12,13]. Recently, a Wnt target gene, *Lgr5/Gpr49*, which encodes an orphan G protein-coupled receptor, was identified as a marker of intestinal stem cells because it marked small columnar cells at the base of the crypt interspersed between Paneth cells [15]. Elegant lineage tracing experiments demonstrated that these few Lgr5+ve cells could reconstitute a villus in an adult mouse upon induction of a cre knock-in allele. The R-spondin (roof plate-specific spondin) family of proteins is comprised of novel secreted proteins, which acts as major agonists and modulators of the Wnt-β-catenin signaling pathway [16,17]. There are four human paralogs (*R-spondin*1–4), each containing a leading signal peptide, two cystein-rich, furin-like domains, and one thrombospondin type 1 domain. Human Rspo1, a 29 kd, 263 amino acid protein, has a specific proliferative effect on intestinal crypt cells [18]. Transgenic expression of Rspo1 in mice resulted in marked hyperplasia of intestinal crypts in both small and large intestine, resulting in abdominal distension [18]. Further experiments demonstrated that Rspo1 prevented mucositis, induced by a chemotherapeutic agent, 5-flurouracil (5-FU), in mice [18] and more recently it was further demonstrated by the same group that Rspo1 protected mice from chemotherapy or radiation-induced oral mucositis [19]. In addition, systemic administration of Rspo1 decreased inflammation and reduced the loss of body weight, diarrhea and rectal bleeding in a mouse model of dextran sulfate sodium-induced colitis [20]. Based upon these findings, we hypothesized that Rspo1 would be radioprotective against RIGS and examined whether Rspo1 was involved in the recovery of the intestine from radiation injury.

## Results

### Serum Rspo1 Levels Are Increased after WBI

RIGS results in part from radiation-induced DNA damage, cell death and/or cell cycle arrest in intestinal crypt cells. Therefore, recovery from RIGS will depend on DNA repair in surviving irradiated crypt clonogens and regeneration of new intestinal progenitor cells. Since Rspo1 enhances the proliferation of intestinal crypt cells, we first examined whether the blood level of Rspo1 is increased after WBI in mice. Immunoblot analysis showed barely detectable levels of endogenous R-spondin1 in the serum of untreated mice. WBI resulted in a two-fold increase in serum Rspo1 concentrations by day 3.5 (Fig 1A and 1B). To evaluate the effect of Rspo1 on RIGS, we injected C57Bl/6J mice with $5\times10^9$ particles of AdRspo1 prior to WBI (Fig 1A). Serum Rspo1 expression increased 6–8 fold in 2 to 3.5 days after AdRspo1 administration and persisted at that level for at least 1 week (Fig 1C). Mice injected with similar doses of the control adenovirus, AdLacZ showed no increase over the base line levels of Rspo1.

### AdRspo1 Improves Survival of Mice after WBI and AIR

In most mammals, including mice, a total-body radiation exposure of more than 10 Gy results in a characteristic gastrointestinal syndrome comprising diarrhea, weight loss and death within 5–14 days [29]. We administered escalating doses of WBI to C57Bl/6J mice to induce RIGS. Exposure to 8.4, 9.4 and 10.4 Gy was lethal in 0%, 20% and 100% of the mice within 14 days, respectively. As the 10.4 Gy dose was uniformly lethal, we administered this dose of WBI to the AdRspo1- and AdLacZ-treated groups to evaluate the radioprotective effects of Rspo1.

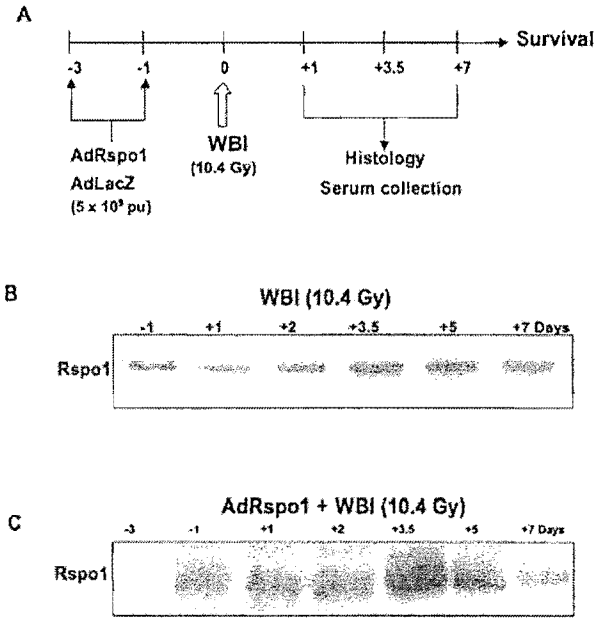


**Figure 1. Time course evaluation of serum Rspo1 expression.** **(A)** Treatment schema: AdRspo1 or AdLacZ ($5\times10^9$ pu) was injected intravenously 3 and 1 day before WBI (10.4 Gy) in C57Bl/6 mice. Animals were followed for survival and histological endpoints. **(B)** Immunoblots of murine serum demonstrating time course evaluation of serum Rspo1 expression after WBI. **(C)** Representative immunoblot of serum Rspo1 levels in C57Bl/6 mice, following treatment with AdRspo1 + WBI.
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Animals receiving WBI had diarrhea and lost body weight within 7 days. In contrast, AdRspo1-treated animals had well-formed stools and maintained body weight after WBI (23.2±0.5 g, AdRspo1 versus 17.26±1.2 g in AdLacZ-treated cohorts; $p<0.0002$). AdRspo1 improved survival of animals exposed to 10.4 Gy WBI significantly ($p<0.003$), with an improvement in median survival time from 10±1.4 days in AdLacZ treated animals to 27±1.6 days in AdRspo1-treated animals. During the first two weeks after WBI, approximately 30% of the animals died in the AdRspo1-treated group, compared with 100% mortality in AdLacZ-treated animals, indicating that Rspo1 protected these animals from RIGS (Fig 2A). The delayed mortality (after 25 days) in the AdRspo1-treated animals was interpreted to be the result of radiation-induced hematopoeitic syndrome. AdRspo1, when administered after the mice were exposed to WBI, could not mitigate the lethal effects of WBI (data not shown).

Since the effects of WBI of 10.4 Gy are secondary to combined hematopoeitic and gastrointestinal syndrome, we wanted to induce primarily a radiation-induced gastro-intestinal injury in mice. We, therefore, administered escalating doses of whole AIR after shielding the thorax, head and neck and extremities, thus protecting the bone marrow. A single fraction of 12, 14 or 16 Gy of AIR was lethal in 100% of mice treated with PBS or AdLcZ by 2 weeks. In contrast, animals treated with AIR + AdRspo1 had well-formed stools and maintained body weight (21.9±0.8, AdRspo1 versus 16.4±0.3 g in AdLacZ-treated cohorts; $p<0.0001$) with only 10% and 30% animals dead at 2 weeks after 12 and 14 Gy of AIR, respectively. There was significant improvement in survival in AdRspo1-treated mice to AIR doses up to 14 Gy ($p<0.002$) (Fig. 2B). There was no radioprotection by AdRspo1 in mice receiving 16Gy AIR.

## AdRspo1 Does Not Protect Tumors from Cytotoxic Effects of AIR

In order to examine whether AdRspo1 could protect tumors from radiation, Balb/c mice with palpable, murine colorectal, CT26 flank tumors were injected with either AdLacz or AdRspo1 virus, followed by 14 Gy AIR, 3 days after viral injection. AdRspo1 did not delay tumor growth compared to AdLacz. As expected, there was significant delay in tumor growth and improved survival only in AdRspo1-treated animals (median survival time 26±2 days) after AIR (Fig 3). Although, AIR reduced tumor growth ($p<0.0001$) but invariably produced 100% mortality of AdLacZ-treated animals. These results demonstrate that Rspo1 could increase the therapeutic ratio of radiation therapy for the treatment of abdominal tumors where it would increase the tolerance of the intestine to irradiation without providing radioprotection to the tumor.

## AdRspo1 Augments Intestinal Crypt Epithelial Cell Proliferation after WBI

Radiation doses of ≥8 Gy induces cell cycle arrest and apoptosis of the crypt epithelial cells within day 1 post-radiation, leading to crypt depletion and a decrease in regenerating crypt colonies by day 3.5 and ultimately villi denudation by day 7 post-radiation exposure [23]. We, therefore, evaluated the histological manifestation of RIGS and the effect of AdRspo1 on RIGS at 1, 3.5 and 7 days, post-WBI. First, we examined whether Rspo1 induces the proliferation of crypt stem cells in mice receiving WBI. As seen in Fig 4, BrdU-labeling cells were vastly amplified in the crypts of AdRspo1+WBI-treated mice, compared to Ad-LacZ+WBI-treated controls at 1 and 3.5 days post-WBI. The percentage of the crypt epithelial cells synthesizing DNA was significantly enhanced after AdRspo1, treatment compared with those administered AdLacZ (AdRspo1, 35±2.27.versus AdLacZ, 22±2.04; $P<0.05$) at 3.5 days following WBI (Fig. 5B). This resulted in an increase in the overall size of the crypts, as determined by measuring crypt depth from the base of the crypt to the crypt-villus junction (Fig. 4 and 5A). A significant increase in the crypt depth in AdRspo1-treated mice compared with AdLacZ-treated mice (AdRspo1, 98.5±5.6 μm versus AdLacZ, 52±3.8 μm; $p<0.001$) was observed, indicating an amplification of the crypt cells after AdRspo1 treatment in irradiated mice (Fig. 4 and 5A). Finally, the intestine in WBI+AdRspo1-treated animals was much longer than those of WBI+AdLacZ-treated animals (38.48±0.9 cm AdRspo1 vs. 33.36±1.1 cm, AdLacZ; $p<0.002$).

## Effect of AdRspo1 on Intestinal Crypt Cell Apoptosis after Radiation Injury

Since ionizing radiation induces apoptosis of intestinal crypt epithelial cells, we performed TUNEL assay to examine apoptosis of crypt epithelial cells, 1 day after WBI. There was a significant ($p<0.001$) decrease in the number of apoptotic nuclei in the jejunal crypts of AdRspo1-treated animals (17±1.2) as compared with the AdLacZ-treated (26.5±1.4) controls (Fig. 4 and 5C), suggesting that Rspo1 might increase the radioresistance of the

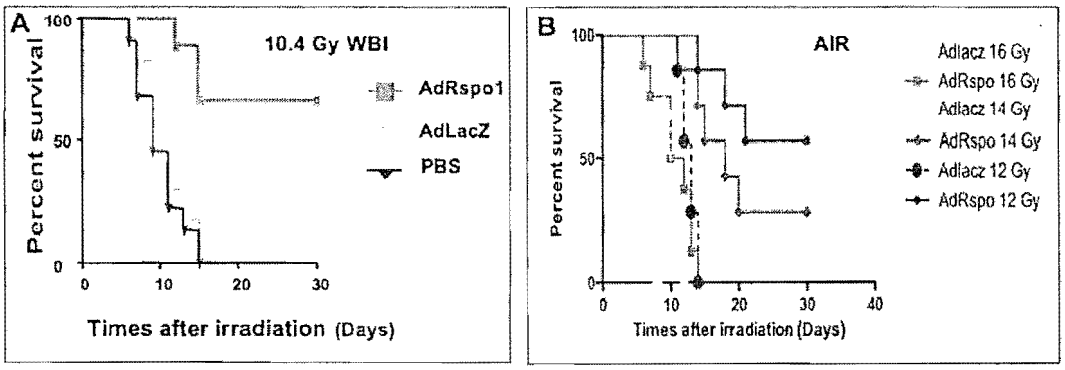



**Figure 2. AdRspo1 treatment protected C57Bl/6 mice from radiation-induced mortality.** Kaplan-Meier survival of C57Bl/6 mice treated with AdRspo1 or AdLacZ prior to WBI (10.4Gy) (Fig 2A) and 12–16 Gy AIR (Fig 2B) . Note a significant ($p<0.003$) increase in median survival in AdRspo1-treated mice with a median survival time of 27±1.6 days, compared to AdLacZ cohorts, 10±1.4 days. With 12–14Gy AIR median survival time for AdLacZ treated animals is 13±1.2 and 11±1.6 days compared to 25±1.3 and 19±1.4 in AdRspo1-treated animals.
doi:10.1371/journal.pone.0008014.g002

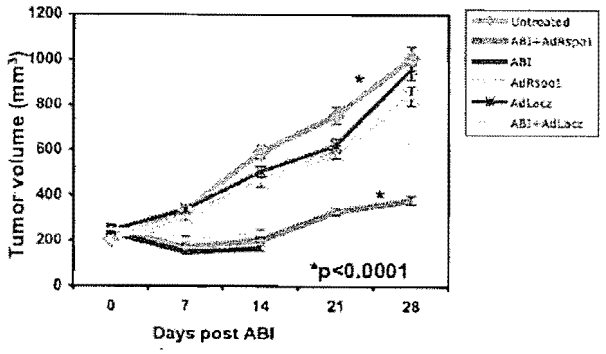



**Figure 3. AdRspo1 treatment has no effect on tumor growth.** Effect of AdRspo1 and AdLacZ treatment on tumor growth rate of Balb/c mice (n=5) irradiated with 14Gy ABI. Significant delay in tumor growth ($p<0.0001$) was observed in ABI groups (Fig A) compared to untreated mice.
doi:10.1371/journal.pone.0008014.g003

intestinal crypt compartment by decreasing radiation-induced apoptosis.

## Crypt Microcolony Assay

Radiation-induced apoptosis of crypt epithelial cells induces compensatory proliferation of intestinal stem cells and transit amplifying cells, resulting in crypt regeneration and clonal growth of damaged intestinal villi. The number of regenerating crypts forming microcolonies between days 3 and 4 after WBI, is a surrogate indicator of the resistance of the intestine to WBI and is correlated with the survival of animals from RIGS. We, therefore, counted the number of regenerative crypts per unit area of intestinal cross section, 3.5 days after exposure to WBI, according to protocols originally described by Withers and Elkind [26]. The number of crypt microcolonies was increased significantly in AdRspo1-treated mouse intestines compared with AdLacZ controls (AdRspo1, 13.8±0.7/μm versus AdLacZ, 8.2±0.5, $p<0.001$, Fig 5D), indicating that Rspo1 induced intestinal crypt regeneration after exposure to WBI.

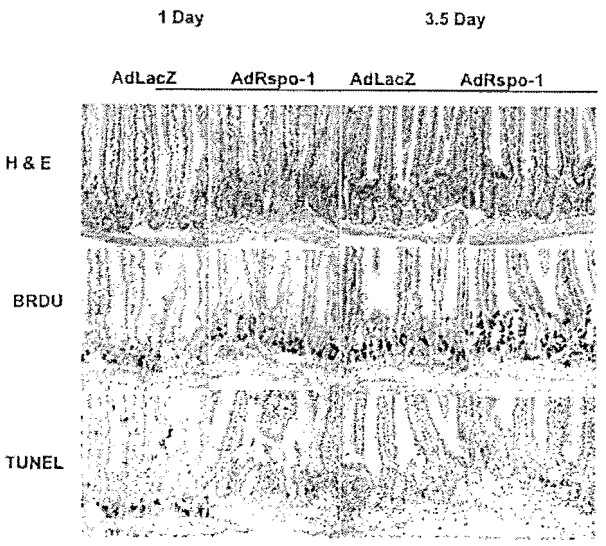



**Figure 4. Histolological assessment of intestine after Irradiation.** H&E staining demonstrates increased crypt depth and increased villi thickness in AdRspo1-treated animals following exposure to WBI. BrdU immunohistochemistry demonstrates higher crypt cell proliferation after AdRspo1 treatment when compared to AdLacZ cohorts. Finally, TUNEL staining demonstrates a decrease in the rate of TUNEL-positive, apoptotic cells in AdRspo1-treated mice post-WBI, when compared to intestinal lumen of AdLacZ-treated mice.
doi:10.1371/journal.pone.0008014.g004

## AdRspo1 Ameliorates Intestinal Malabsorption Syndrome in RIGS

To evaluate the functional regeneration and absorptive capacity of the intestine, animals from various treatment cohorts were fed xylose solution following exposure to WBI. Since xylose is not metabolized in the body, serum xylose levels are a good indicator of the intestinal absorptive capacity. As expected, there was a consistent reduction in xylose absorption in AdLacZ-treated mice (33.5±7.5 g/ml), 7 days after WBI. In contrast, there was a significant recovery of xylose absorption in AdRspo1-treated mice (75±3.8 g/ml; $p<0.002$) at this time point. Xylose absorption continued to improve in the AdRspo-1 treated animals up to 10 days post-WBI (Fig. 6), indicating quick restitution of the intestinal villi.

## β-Catenin Localization in Nuclear and Cytosolic Fraction

Recent reports indicate that the R-spondin proteins activate β-catenin signaling [20,30], which is critical in maintaining intestinal homeostasis [13]. Under resting condition, β-catenin is present in the cytoplasm. Phosphorylation of β-catenin (by GSK-3 kinase) targets the proteins to to proteosomes where it is degraded. Wnt activation inhibits GSK-3 kinase phosphorylation of β-catenin, preventing β-catenin degradation and allowing for its translocation from the cytoplasm to the nucleus. In the nucleus, β-catenin binds to and activates the TCF/LEF transcription factor complex to induce the expression of wnt-pathway genes, such as, EphB2, EphB3, TCF4 and LEF1. We, therefore, examined the relative levels of β-catenin protein in the cytoplasm and nucleus of intestinal epithelial cells isolated from the two cohorts of animals that received WBI. Immunoblot analysis demonstrated a slight increase in nuclear β-catenin levels, 1 day after WBI in AdLacZ-treated mice (Fig 7A). In contrast, the nuclear/cytosolic ratio of β-catenin was much higher in Ad-Rspo1-treated mice in basal conditions (day −1, Fig 7B), which further increased by 2–4 folds the value of AdLacZ-treated animals, with a peak around 3.5 days upon exposure to WBI (Fig 7A and B). Immunohistochemistry confirmed an increase in nucelar β-catenin staining in the crypt progenitor cells in AdRspo1-treated animals, suggesting that Rspo1 enhanced stabilization and nuclear translocation of β-catenin in crypt cells in these animals (data not shown).

## AdRspo1 Amplifies the Number of Lgr5-Positive Crypt Stem Cells

Immunohistochemical staining of murine jejunum crypts showed a significant increase in the number of Lgr5-expressing intestinal stem cells at crypt columnar base in the AdRspo1-treated mice (Fig. 8). Three and a half days after exposure to WBI, while the Lgr5+ve crypt stem cells decreased in AdLacZ-treated mice, these cells remain amplified in AdRspo1-treated mice, suggesting an expansion of the crypt stem cell compartment contributed to the protection from RIGS.

## Real Time PCR of the Expression of β-Catenin Target Genes

The expression of target genes of the β-catenin pathway in these animals was determined by realtime PCR. The mRNA levels of

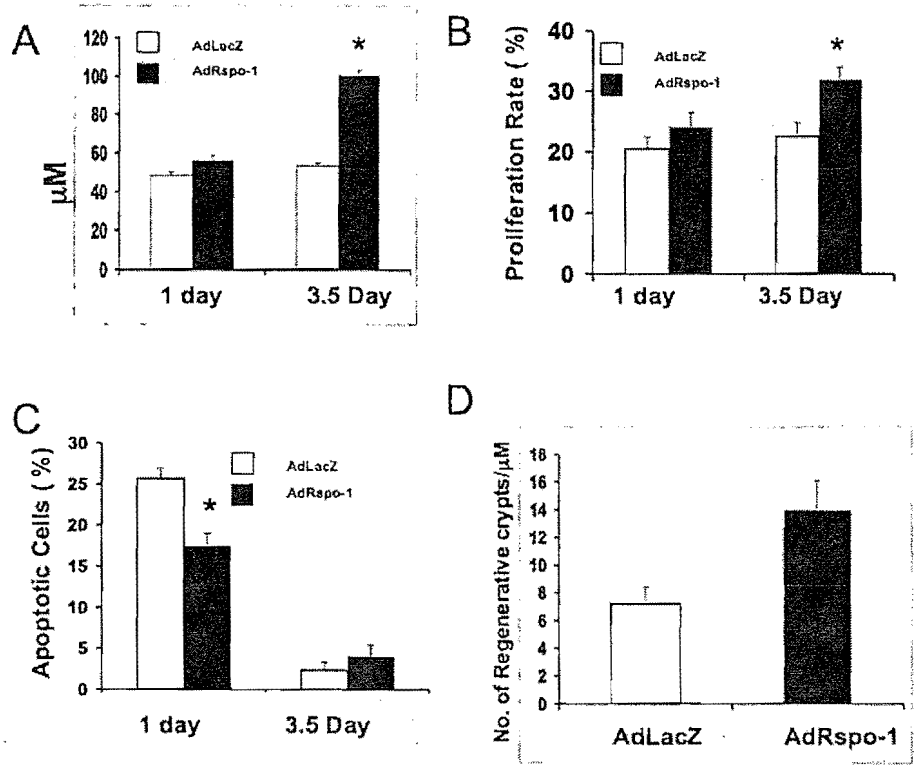



**Figure 5. AdRspo1 increases the number of regenerative crypts in irradiated mice.** Effect of AdRspo1 and AdLacZ treatment on intestinal crypt depth (A), proliferation rate (B), apoptotic cells (C) at 1day and 3.5 days after WBI and the number of regenerative crypts (D) at 3.5 days after WBI. A representative sampling of thirty crypts was assessed for each treatment group.
doi:10.1371/journal.pone.0008014.g005

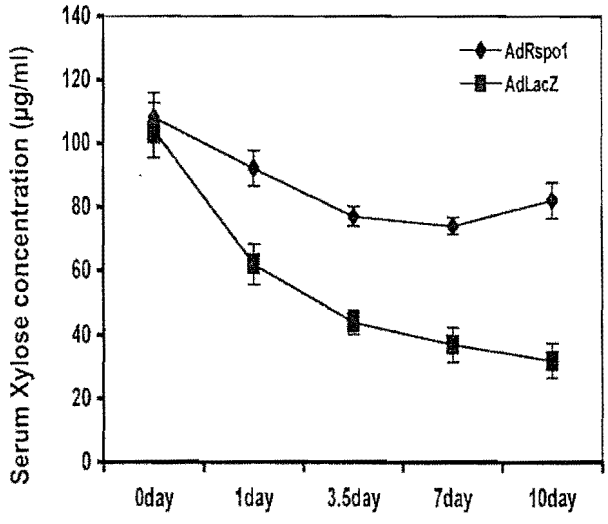



**Figure 6. Xylose absorption assay.** A time course study (1–10dys) showed significant recovery ($p<0.002$) of xylose absorption at 3.5 to 7 days in AdRspo1-treated cohorts, when compared to AdLacZ controls, thereby indicating the functional regeneration of intestine after radiation injury. AdLacZ-treated animals were incapable of demonstrating adequate xylose absorption after radiation injury, further contributing to animal mortality.
doi:10.1371/journal.pone.0008014.g006

EphB2 and EphB3 were found to be increased by 1.85 fold and 4.8 fold, respectively in AdRspo1-treated animals exposed to WBI, as compared with AdLacZ-treated cohorts. The mRNA levels of the β-catenin target genes, TCF4 and Lef1 were also upregulated approximately 2.5 fold in response to Rspo1 after irradiation while the expression of TCF1 and TCF3 were unchanged.

## Discussion

The gastro-intestinal (GI) system is a major target for the somatic injuries associated with radiation and chemotherapy. Because of this, RIGS is an important cause of host vulnerability whether in medical therapeutics or in nuclear accidents or terrorism. Rspo1 was originally identified as a growth factor for intestinal crypt cells in a mouse transgenic model [18]. In a mouse xenograft model of human colon carcinoma, CT26, treatment with Rspo1 reduced the mucositis, diarrhea and weight loss caused by the chemotherapeutic agent, 5-flurouracil (5-FU), without affecting its antitumor effect [18]. Furthermore, systemic administration of Rspo1 decreased the histological and clinical manifestation of dextran sulfate sodium-induced colitis [20] and chemotherapy and radiation-induced oral mucositis [19] in mice. These data suggested that Rspo1 might play an important role in maintaining intestinal mucosal integrity.

Zhao et al demonstrated that prophylactic treatment with recombinant RSpo1 protein increased the mucosal thickness and reduced ulceration in the oral mucosa after irradiation and chemotherapy, presumably by increasing the proliferation of the mucosal epithelium in the basal layer of the tongue [19].

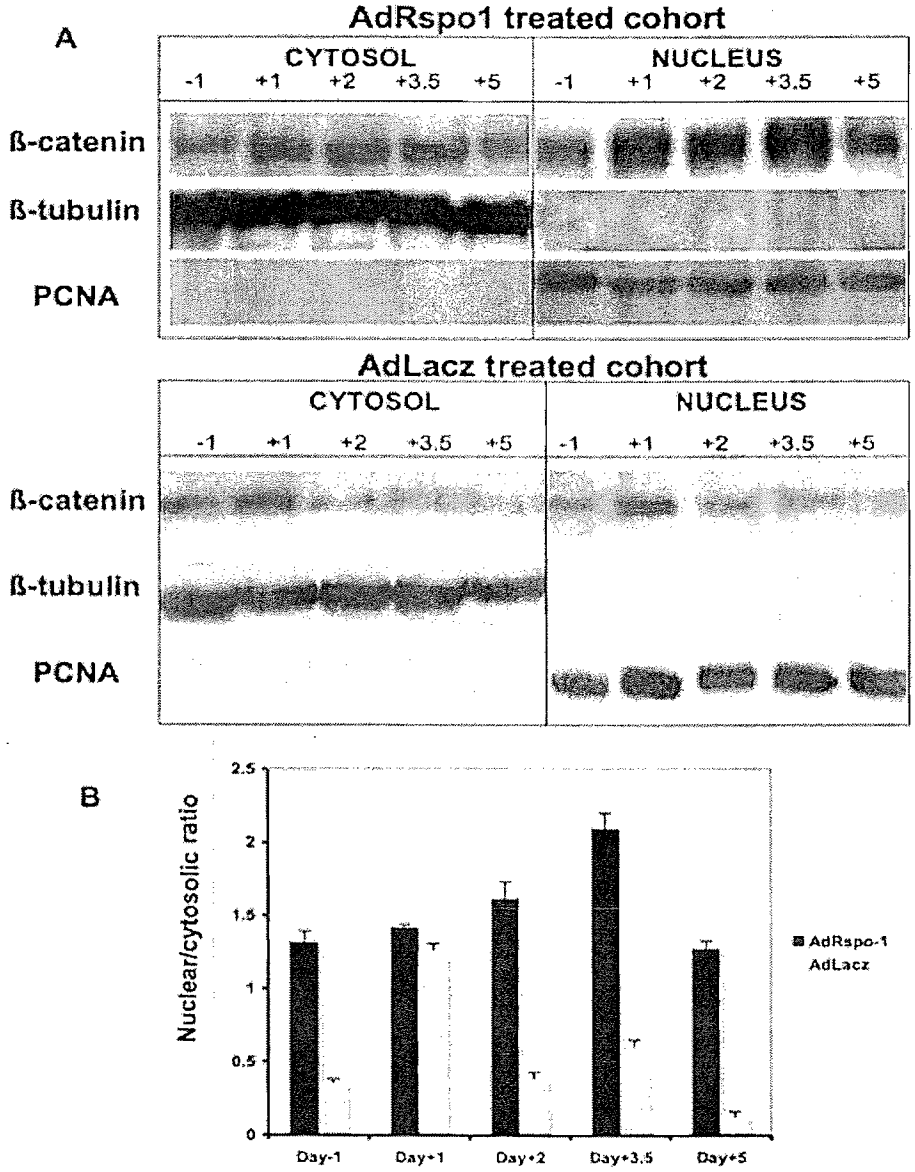



**Figure 7. AdRspo1 treatment induces β-catenin activation in irradiated crypts.** Representative immunoblot (**Fig. 7A**) and densitometric analysis (**Fig. 7B**) of nuclear/cytosolic ratios of β-catenin from AdRspo1 and AdLacZ treated cohorts after WBI(10.4Gy). Nuclear fraction purity was validated by the absence of β-tubulin, while the purity of the cytosolic fraction was evaluated by the absence of PCNA (Fig. 7A). A continuous decline in nucear/cytosolic ratios of β-catenin was predominate in samples from irradiated AdLacZ cohorts. This is further supported by the densitometric analysis of β-catenin expression (Fig. 7B) from the nuclear/cytosolic ratio demonstrating the significant differences in AdRspo1 when compared to AdLacZ treated mice prior to (Day −1) until Day +5 post WBI.
doi:10.1371/journal.pone.0008014.g007

Although, Rspo1 protected radiation-induced oral mucosal injury, the effect of Rspo1 in the functional regeneration of the intestinal mucosal epithelium and amelioration of RIGS has not been studied. In this report, we demonstrate that Rspo1 is induced after exposure to WBI as a physiological response to irradiation exposure. Systemic administration of an adenovirus expressing recombinant Rspo1 amplified the Lgr5+ve intestinal crypt stem cell population and ameliorated RIGS and improved survival of mice. The effect of AdRspo1 on the regeneration of the intestinal mucosa after irradiation was manifested physically by significantly higher intestinal length and diameter, increased crypt depth and proliferative index, decreased crypt epithelial apoptosis, increased regenerative crypt microcolonies and maintenance of the villi length. This improved clinical, gross, and histopathological effects on the small intestine after WBI and AIR in AdRspo1-treated mice were physiologically manifested by a marked and progressive restoration of the normal absorptive function of the intestine, as measured by xylose absorption test.

R-spondins are a family of secreted proteins that are expressed in the small intestine, kidney, prostate, adrenal gland and pancreas

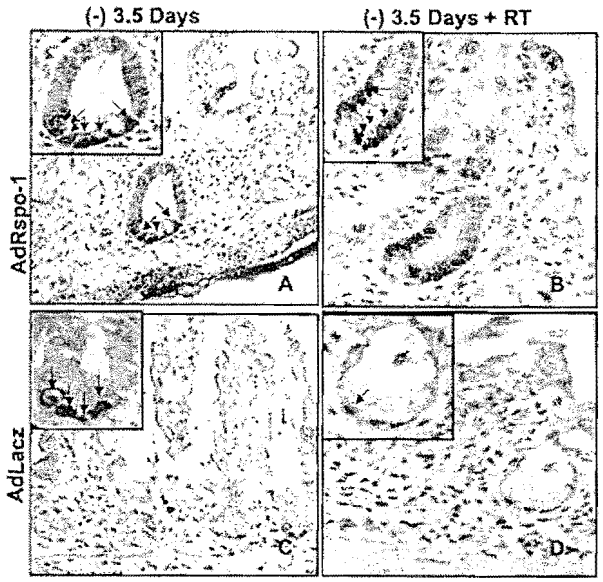



**Figure 8. AdRspo1 treatment increases the number of Lgr5-positive intestinal stem cells in irradiated crypts.** Immunohistochemical staining of Lgr5 in murine jejunum crypts at 3.5 days prior and after WBI. There was an increase in the number of Lgr5 postive cells at crypt columnar base in AdRspo1 treated cohorts when compared to AdLacZ (magnification 60x; *arrows*).
doi:10.1371/journal.pone.0008014.g008

[18] and are potent activators of the Wnt-β-catenin pathway [31]. Rspo1 has been demonstrated to bind with high affinity to the Wnt co-receptor, LRP6, to induce phosphorylation, stabilization and nuclear translocation of cytosolic β-catenin, thereby activating TCF/β-catenin-dependent transcriptional responses in intestinal crypt cells [32]. Our results suggest that the induction of Rspo1 after TBI may be an important protective pathway in the repair of intestinal injury in RIGS. In our experiments, Rspo1 could not prevent the mortality of the animals from the hematopoeitic syndrome, since all animals receiving WBI + AdRSpo1 were dead by 25–28 days. However, Rspo1 protected the death from GI syndrome, even with higher doses of AIR (12–14 Gy). Rspo1 likely promotes protection of RIGS through a combination of reduced radiation-induced apoptosis (i.e. increased cell survival), increased crypt cell proliferation with enhanced crypt regeneration, and rapid restoration of the structure and absorptive function of the villi. On a cellular level, AdRspo1 treatment increased the levels of nuclear β-catenin and wnt target gene expression in irradiated crypt cells. Notable among the wnt target genes that are induced in AdRspo1-treated animals are Tcf4 and Lef1, two genes that are responsible for intestinal epithelial cell proliferation and maintenance of homeostasis. Similarly, EphB2 and EphB3 are induced and could mediate crypt cell proliferation, differentiation and cell positioning along the crypt villus axis, following WBI. Furthermore, the number of Lgr5+ve crypt base columnar cells, resembling the intestinal stem cells as described by Cheng and Leblond [8], was amplified in AdRspo1+WBI-treated mice. These data, in conjunction with the histological findings of an increase in crypt regeneration and improved intestinal restitution after WBI in mice treated with AdRspo1, as compared to AdLacZ, indicates that Rspo1 mediates induction of an intestinal regenerative process, possibly as a salvage mechanism, following exposure to WBI. Furthermore, compared with AdLacZ-treated controls, pretreatment with AdRspo1 reduced WBI-associated intestinal crypt cell apoptosis. Since the wnt/β-catenin signaling has been postulated to promote radioresistance of mammary epithelial stem cells [33], Rspo1 might also confer radioprotection to crypt progenitor cells by stimulating Wnt-β-catenin signaling in RIGS.

Several growth factors and cytokines including KGF, TGFbeta, TNFα, PGE2, IL11 [34,35,36,37] have been shown to protect intestine from radiation or other cytotoxic injury by increasing the crypt cell proliferation and survival. While growth factors, such as, bFGF could minimize the radiation induced intestinal damage by reducing apoptosis [38,39]. To our knowledge, this is the first demonstration of the salutary effect of Rspo1 in the context of radiation injury of the intestine where it played a protective role by amplifying the stem cell population along with inhibition of radiation induced apoptosis in crypt. Since, Rspo1 has no protective effect on tumors during chemotherapy [18] and radiation therapy (Fig 3), systemic use of Rspo1, by protecting the normal intestinal tissue, may increase the therapeutic ratio of chemoradiation therapy in patients undergoing abdominal irradiation for GI malignancies. While the mechanism(s) associated with preserving structural regeneration and function ensures the potential prophylactic and salvage role of hRspo1 in rescuing the absorptive capacity of intestine, further studies are warranted to evaluate its potential as a therapy for RIGS in combination with other mitigating agents by reversing radiation-induced injury of the intestine.

## Materials and Methods

### Animals

Five- to 6-weeks-old male C57Bl/6 mice (NCI-Fort Dietrich, MD) were maintained in the animal maintenance facilities and all animal studies were performed under the guidelines and protocols of the Institutional Animal Care and Use Committee of the Albert Einstein College of Medicine.

### Adenovirus Construction and Administration

Since recombinant Rspo1 was not available to us, we constructed an adenovirus (AdRspo1) expressing human R-spondin1 protein and used adenoviral gene transfer for proof-of-concept experiments. Human R-spondin1 cDNA (Origene, Rockville, MD) was subcloned in pShuttle-2 (Clonetech, Mountain View, CA), followed by ligation into the Adeno-X viral DNA according to protocols described in the Adeno-XTM expression system (Clonetech, Mountain View, CA). The recombinant adenoviral vector was linearized with Pac-1 and transfected in 293 kidney cells (ATCC, Manassas, VA) using Lipofectamine plus (Invitrogen, Carlsbad, CA), according to the manufacturer's protocol until a cytopathetic effect (CPE) appeared. 293 cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Atlanta Biologicals, Lawrenceville, Ga), and supplemented with 0.1 mM nonessential amino acids (Invitrogen, Carlsbad, CA), 100 μg/ml streptomycin (Invitrogen, Carlsbad, CA) and 100 units/ml penicillin (Invitrogen, Carlsbad, CA). Viral lysates were amplified and subjected to $CsCl_2$ gradient centrifugation to purify the recombinant AdRspo1 adenovirus as described[21]. The adenovirus expressing the ß-galactosidase gene of E. coli (AdLacZ) was used as a control adenovirus in these experiments. All viruses were stored as $5\times10^{10}$ particles/ml of glycerol buffer.

$5\times10^{9}$ particles of AdRspo1 or AdLacZ (adenovirus expressing *β-galactosidase* gene of E. coli as control) were injected intravenously via tail vein, 1–2 times at 3 and/or 1 day before whole body irradiation (WBI). Viral lysates were amplified and subjected to

$CsCl_2$ gradient centrifugation to purify the recombinant AdRspo1 adenovirus as described elsewhere [21,22]. All viruses were stored as $5\times10^{10}$ particles/ml of glycerol buffer.

## Irradiation Procedure

Whole-body irradiation (WBI) was performed on anesthetized mice (intraperitoneal ketamine and xylazine 7:1 mg/ml for 100 l/mouse) using a Shephard $^{137}$Cs -ray irradiator at a dose rate of 236cGy/min following biosafety guidelines of Albert Einstein College of Medicine. Initially a dose response (8–10.4 Gy) of WBI demonstrated that C57Bl/6 mice receiving 10.4 Gy died within two weeks, suggesting death from RIGS. Thereafter, protection experiments with AdRspo1 were performed with 10.4 Gy. Since 10.4 Gy WBI can induce both hematopoeitic and gastrointestinal injury, we also administered escalating doses (12–16 Gy) of whole abdominal irradiation (AIR) after shielding the thorax, head and neck and extremities and protecting a significant portion of the bone marrow, thus inducing predominantly RIGS.

## Irradiation of Abdominal Tumors

Balb/c mice were injected with $1\times10^6$ CT26 colon cancer cells (ATCC, Manassas, VA) on the flank. Ten days after tumor inoculation, animals with palpable tumors received an intravenous injection of AdRspo1 ($1\times10^{11}$ particles), followed by whole AIR of 14Gy by Mark I$^{137}$ Cs source a day later.

## Detection of Rspo1 Expression in Blood

Blood was drawn from the retro-orbital plexus and serum was isolated by centrifugation at 10,000 rpm for 5 min. Serum protein concentration was determined by Bradford assay kit (Bio-Rad Laboratories, Hercules, CA). Approximately 100 µg of protein was subjected to 14% SDS-PAGE, followed by electroblotting onto polyvinylidene difluoride membranes. The blot was blocked with 5% skim milk in Tris-buffered saline (10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.05% Tween 20) followed by incubation with primary antibody (1:200 dilution), goat polyclonal anti mouse Rspo1 (R & D Systems, Minneapolis, MN), and then with secondary antibody (1:500 dilution), horseradish peroxidase (HRP) conjugated bovine anti-goat antibody (Santa-Cruz Biotechnology, Inc., Santa Cruz, CA). The blots were developed using Enhanced Chemiluminence assay (Amersham Pharmacia Biotech, Inc, Piscataway, NJ).

## Histology

Since radiation doses greater than 8 Gy induces cell cycle arrest and apoptosis of the crypt epithelial cells within day 1 post-radiation, resulting in a decrease in regenerating crypt colonies by day 3.5 and ultimately villi denudation by day 7 post-radiation exposure [23], we sacrificed animals when moribund or at 1, 3.5 and 7 days after WBI or AIR for time course experiments and intestine were harvested for histology. The intestine of each animal was dissected, washed in PBS to remove intestinal contents and the jejunum was fixed in 10% neutral buffered formalin prior to paraffin embedding. Tissue was routinely processed and cut into 5 µm sections for hematoxylin and eosin and immunohistochemical staining. All haemotoxylin and eosin (Fisher Scientific, Pittsburgh, PA) staining was performed at the Histology and Comparative Pathology Facility in the Albert Einstein Cancer Center. A total of 30 crypts were examined per animal for all histological parameters.

## Crypt Proliferation Rate

To visualize villous cell proliferation, each mouse was injected intraperitoneally with 120 mg/kg BrdU (Sigma-Aldrich, USA) 2–4 hrs prior to sacrifice and mid-jejunum was harvested for paraffin embedding and BrdU immunohistochemistry. Tissue sections were routinely deparaffinized and rehydrated through graded alcohols and incubated overnight at room temperature with a biotinylated monoclonal BrdU antibody (Zymed, South Francisco, CA). Nuclear staining was visualized using Streptavidin-peroxidase and diaminobenzidine (DAB) and samples were lightly counter-stained with hematoxylin. Jejunum from mice, not injected with BrdU, was used as a negative control. Murine crypts were identified histologically according to the criteria established by Potten et al [24]. Digital photographs of crypts were taken at high (400–600X) magnification (Zeiss AxioHOME microscope) and crypt epithelial cells (paneth and non-paneth) intestinal sections were examined using ImageJ software and classified as BrdU positive if they grossly demonstrated brown-stained nuclei from DAB staining or as BrdU negative if they were blue stained nuclei. The proliferation rate was calculated as the percentage of BrdU positive cells over the total number of cells in each crypt.

## Determination of Crypt Depth

Crypt depth was independently and objectively analyzed and quantitated in a blind fashion from coded digital photographs of crypts from H&E stained slides using ImageJ 1.37 software to measure the height in pixels from the bottom of the crypt to the crypt-villus junction. This measurement in pixels was converted to length (in µm) by dividing with the following a conversion factor (1.46 pixels/µm).

## Detection of Apoptosis In Situ

Apoptotic cells were detected *in situ* by performing TUNEL (TdT-mediated digoxigenin labeled dUTP nick end labeling) staining. Briefly, paraffin embedded sections were de-paraffinized, rehydrated through graded alcohols and stained using an ApopTag kit (Intregen Co, Norcross, Georgia). The apoptotic rate in crypt cells was quantified by counting the percent of apoptotic cells in each crypt with analysis restricted to "intact" longitudinal crypt sections in which the base of the crypt was aligned with all the other crypt bases and the lumen [3,24].

## In Vivo Crypt Microcolony Survival Assay

Intestinal crypt survival was measured using a modification of microcolony assay [25,26]. A regenerative crypt comprised of tightly compacted and occasionally multi-layered large epithelial cells with a highly basophilic cytoplasm and large nuclei. The viability of each surviving crypt was confirmed by immunohistochemical detection of BrdU incorporation into five or more epithelial cells within each regenerative crypt. A minimum of four complete cross-sections was scored for each mouse and representative kinetic data were obtained from two mice in each group. Because the size of the regenerating crypt may not be the same for each treatment group, the number of surviving crypt per cross section was normalized to crypt size. Surviving crypts were defined as containing 10 or more adjacent chromophilic non-Paneth cells, a Paneth cell and lumen [25].

## Immunohistochemistry

For immunohistochemical staining of formalin-fixed, paraffin-embedded tissue sections, endogenous peroxidase activity was blocked for 30 min with methanol containing 0.3% $H_2O_2$. Antigen retrieval was performed by heating slides in pH 6.0 citrate buffer at 100°C for 20 min in a microwave oven at 500 watts. Non-specific antibody binding was blocked for 20 minutes by incubation with 10% normal rabbit serum. Sections were

incubated with primary monoclonal antibody against β-catenin diluted 1:200, and Lgr5 diluted 1:250 (Transduction Laboratories, Lexington, KY), either 1 hr at room temperature or overnight at 4°C. The primary antibody was visualized using a streptavidin-biotin-peroxidase (ABC) kit (DAKO, Carpinteria, CA) with diaminobenzidine tetrahydrochloride (3,3′-diaminobenzidine) as the chromogen. These sections were then lightly couterstained by haematoxylin (Fisher Scientific, Pittsburg, PA).

## Isolation of Intestinal Epithelial Cells

Intestinal epithelial cells were prepared from the jejunum of adult male C57Bl6 mice by modification of the protocol described by Weiser and Ferraris [27]. Briefly, mice were anaesthetized and a catheter was inserted into the intestine through an incision in the most proximal part of duodenum. A second incision was made just proximal to the cecum and the entire small intestine was perfused with ice-cold PBS and then flushed twice with ice-cold PBS plus 1 mM dithiothreitol (DTT). The duodenum and ileum were discarded and the entire jejunum was tied at the distal end and filled to distension with isolation citrate buffer (0.9% NaCl, 1.5 mM KCl, 27.0 mM Na Citrate, 8.0 mM $KH_2PO_4$ and 5.6 mM $Na_2HPO_4$, pH 7.3) heated to 37°C for 15 mins. After incubation, the jejunum was emptied and filled with 5 ml ethylene diamine tetra acetic acid (EDTA) buffer (0.9% NaCl, 8 mM $KH_2PO_4$, 5.6 mM $Na_2HPO_4$, 1.5 mM $Na_2$-EDTA, pH 7.6, plus 0.5 mM DTT and 0.23 mM PMSF) (Sigma Aldrich, St. Louis, MO). Each jejunum was then physically manipulated and tapped allowing the cells to separate from the interior surface. The jejunum was finally rinsed twice with 5 ml of EDTA buffer and all the fluid containing epithelial cells was collected, centrifuged at 300×g (Sorvell Rc5c) for 5 min, washed twice with 20 mL of balanced salt solution (BSS) containing 135 mM NaCl, 4.5 mM KCl, 5.6 mM glucose, 0.5 mM $MgCl_2$, 10 mM HEPES and 1.0 mM $CaCl_2$, pH 7.4, and the cells suspended in 2 mL of the same solution. Cell numbers were determined with hemocytometer and viABIlity (>90±5%) was assessed using trypan blue exclusion.

## Detection of β-Catenin Expression in Intestinal Cells by Immunoblot

Intestinal epithelial cells were isolated from the jejunum of AdRspo1- and AdLacZ-treated mice by modification of the protocol described by Weiser and Ferraris [27] as described in supplement. Isolated cells were fractionated as cytosolic and nuclear part by Nuclear/Cytosol Fractionation kit (Biovision Incorporated, Mountain View, California), according to the manufacturer's protocol and then subjected to immunoblot to analyze the β-catenin expression using mouse monoclonal antibody β-catenin (BD Bioscience, San Jose, CA). The immunoblot was developed and signal was detected by Chemiluminance assay (Amersham Pharmacia Biotech Inc, Piscataway, NJ). Purity of nuclear and cytosolic fractions was determined by the relative absence of β-tubulin and PCNA, respectively.

## RNA Isolation

Isolated murine intestinal epithelial cells were lysed using RLT buffer from RNeasy Mini Kit (Qiagen, Valencia, CA) and 1% betamercaptoethanol mix. Qiagen's protocol for the RNeasy Mini Kit with on-column DNA digestion was used to isolate RNA from the lysates. The RNA samples were stored at −80°C prior to use.

## Realtime PCR of β-Catenin Target Genes

To analyze the involvement of β-catenin downstream pathway in Rspo1 mediated intestinal repair mRNA levels of different β-catenin target genes in intestinal epithelial cells from from AdRspo1 and AdLacZ treated mice before and after WBI (10.4 Gy) were analyzed by real time PCR. cDNA was synthesized using the SuperScript™ First-Strand Synthesis System from Invitrogen. Realtime PCR was performed in Light Cycler real time PCR machine (Bio Rad Laboratories, Hercules, CA) using the ABsolute QPCR SYBER Green Mix (ABgene, Rochester, USA). The conditions followed the standard ABgene protocol with the exception for the annealing and extension step, where a temperature of 55°C for EphB2 and EphB3, 57°C for Tcf4, and 54°C for Lef1 were used for 30 seconds followed by 30 seconds at 72°C. To check for primer amplification specificity, a melting curve was generated at the end of the PCR and different samples containing the same primer pair showed matching amplicon melting temperatures. The gene sequences of β-catenin target genes were obtained from the Ensembl mouse genome database (http://www.ensembl.org/Mus_musculus/index.html) and the primers were designed using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Any primer pair generated with Primer3 was checked for gene specificity using the nucleotide-nucleotide BLAST database (http://130.14.29.110/BLAST/). The primer pairs used were as follows:

Beta actin: sense primer 5′ TGTACCCAGGCATTGCTGAC 3′ and anti-sense primer 5′ ACAGTGAGGCCAGGATGGAG 3′;

Ephb2: Sense primer 5′ AAGATGGGCCAGTACAAGGA 3′ and anti-sense primer 5′ CCAGCTAGAGTGACCCCAAC 3′;

Ephb3: sense primer 5′ TGGGACGGTACAAGGAGAAC 3′ and anti-sense primer 5′ TCATGTCCTGAATGCTGCTC 3′;

Tcf4: sense primer 5′ GGCGTTGGACAGATCACC 3′ and anti-sense primer 5′ GGTGAAGTGTTCATTGCTGTACTG 3′;

Lef1: sense primer 5′ AGACACCCTCCAGCTCCTGA 3′ and anti-sense primer 5′ CCTGAATCCACCCGTGATG 3′.

## Xylose Absorption Assay

To quantify intestinal absorption as a physiological indicator of mucosal barrier integrity in AdRspo1-, and AdLacZ-treated mice (n = 5/group) after WBI, a xylose uptake assay was performed, at various time points (1, 3.5, 7 and 10 days) after irradiation. A 5% w/v solution of D-xylose (100l/mouse) in deionized water was administered orally by feeding tube and 2 hrs post administration of D-xylose animals were sacrificed and blood samples collected using heparinized blood collection tubes (BD Biosciences, San Jose, CA). For determination of plasma D-xylose concentration a modified micromethod as reported by Eberts et al. was used [28]. One mL phloroglucinol (1,3,5-trihydroxybenzene, Sigma Chemical Co., St. Louis, MO) reagent (0.5 g of phloroglucinol, 100 mL glacial acetic acid and 100 mL of conc. HCL) was added to 10L of plasma. This solution was heated to 100°C in a water bath for 4 min to allow optimum color development. After equilibration to room temperature, sample absorption was determined with the aid of a spectrophotometer set at a wavelength of 554 nm.

## Kaplan-Meier Survival Curve Analysis

The effect of irradiation and concomitant Rspo1 on mice survival/mortality was analyzed by kaplan-Meier as a function of radiation (WBI and/or AIR) dose using Sigma-Plot and Graphpad Prism-4.0 software for Mac.

## Statistical Analysis of Digital Images

Sampling regions were chosen at random for digital acquisition for data quantitation. Digital image data was evaluated in a blinded fashion as to any treatment. A total of thirty to sixty crypts from two mice/treatment group were used for each data point. A two-sided student's t-test was used to determine

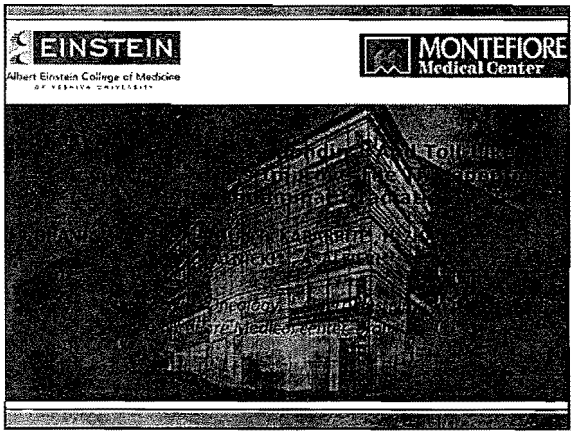
significant differences between AdLacZ and AdRspo1 treated mice ($P<0.05$) with representative standard errors of the mean (SEM).

## Author Contributions

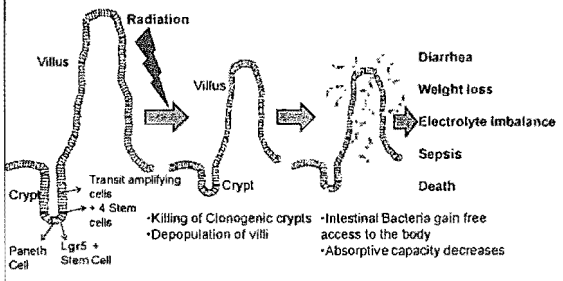
Conceived and designed the experiments: PB NRC JRC CG. Performed the experiments: PB SS LL. Analyzed the data: PB SS RK RSS. Contributed reagents/materials/analysis tools: CG. Wrote the paper: PB SS CG. Edited the paper: AAA.

## References


1. Marshman E, Booth C, Potten CS (2002) The intestinal epithelial stem cell. Bioessays 24: 91–98.
2. Potten CS (1998) Stem cells in gastrointestinal epithelium: numbers, characteristics and death. Philos Trans R Soc Lond B Biol Sci 353: 821–830.
3. Potten CS, Merritt A, Hickman J, Hall P, Faranda A (1994) Characterization of radiation-induced apoptosis in the small intestine and its biological implications. Int J Radiat Biol 65: 71–78.
4. Zimmerer T, Bocker U, Wenz F, Singer MV (2008) Medical prevention and treatment of acute and chronic radiation induced enteritis–is there any proven therapy? a short review. Z Gastroenterol 46: 441–448.
5. Hauer-Jensen M (1990) Late radiation injury of the small intestine. Clinical, pathophysiologic and radiobiologic aspects. A review. Acta Oncol 29: 401–415.
6. Coia LR, Myerson RJ, Tepper JE (1995) Late effects of radiation therapy on the gastrointestinal tract. Int J Radiat Oncol Biol Phys 31: 1213–1236.
7. Potten CS, Kovacs L, Hamilton E (1974) Continuous labelling studies on mouse skin and intestine. Cell Tissue Kinet 7: 271–283.
8. Cheng H, Leblond CP (1974) Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. III. Entero-endocrine cells. Am J Anat 141: 503–519.
9. Potten CS, Morris RJ (1988) Epithelial stem cells in vivo. J Cell Sci Suppl 10: 45–62.
10. Francois S, Bensidhoum M, Mouiseddine M, Mazurier C, Allenet B, et al. (2006) Local irradiation not only induces homing of human mesenchymal stem cells at exposed sites but promotes their widespread engraftment to multiple organs: a study of their quantitative distribution after irradiation damage. Stem Cells 24: 1020–1029.
11. Monti P, Wysocki J, van der Meeren A, Griffiths NM (2005) The contribution of radiation-induced injury to the gastrointestinal tract in the development of multi-organ dysfunction syndrome or failure. BJR Suppl 27: 89–94.
12. Inagaki-Ohara K, Yada S, Takamura N, Reaves M, Yu X, et al. (2001) p53-dependent radiation-induced crypt intestinal epithelial cells apoptosis is mediated in part through TNF-TNFR1 system. Oncogene 20: 812–818.
13. Gregorieff A, Clevers H (2005) Wnt signaling in the intestinal epithelium: from endoderm to cancer. Genes Dev 19: 877–890.
14. Pinto D, Gregorieff A, Begthel H, Clevers H (2003) Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. Genes Dev 17: 1709–1713.
15. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, et al. (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449: 1003–1007.
16. Kim KA, Wagle M, Tran K, Zhan X, Dixon MA, et al. (2008) R-Spondin Family Members Regulate the Wnt Pathway by a Common Mechanism. Mol Biol Cell, 19(6): 2588–96.
17. Nam JS, Turcotte TJ, Smith PF, Choi S, Yoon JK (2006) Mouse cristin/R-spondin family proteins are novel ligands for the Frizzled 8 and LRP6 receptors and activate beta-catenin-dependent gene expression. J Biol Chem 281: 13247–13257.
18. Kim KA, Kakitani M, Zhao J, Oshima T, Tang T, et al. (2005) Mitogenic influence of human R-spondin1 on the intestinal epithelium. Science 309: 1256–1259.
19. Zhao J, Kim KA, De Vera J, Palencia S, Wagle M, et al. (2009) R-Spondin1 protects mice from chemotherapy or radiation-induced oral mucositis through the canonical Wnt/beta-catenin pathway. Proc Natl Acad Sci U S A 106: 2331–2336.
20. Zhao J, de Vera J, Narushima S, Beck EX, Palencia S, et al. (2007) R-spondin1, a novel intestinotrophic mitogen, ameliorates experimental colitis in mice. Gastroenterology 132: 1331–1343.
21. Chartier C, Degryse E, Gantzer M, Dieterle A, Pavirani A, et al. (1996) Efficient generation of recombinant adenovirus vectors by homologous recombination in Escherichia coli. J Virol 70: 4805–4810.
22. Kuo CJ, Farnebo F, Yu EY, Christofferson R, Swearingen RA, et al. (2001) Comparative evaluation of the antitumor activity of antiangiogenic proteins delivered by gene transfer. Proc Natl Acad Sci U S A 98: 4605–4610.
23. Potten CS, Booth C, Pritchard DM (1997) The intestinal epithelial stem cell: the mucosal governor. Int J Exp Pathol 78: 219–243.
24. Potten CS (1992) The significance of spontaneous and induced apoptosis in the gastrointestinal tract of mice. Cancer Metastasis Rev 11: 179–195.
25. Potten CS, Rezvani M, Hendry JH, Moore JV, Major D (1981) The correction of intestinal microcolony counts for variation in size. Int J Radiat Biol Relat Stud Phys Chem Med 40: 321–326.
26. Withers HR, Elkind MM (1970) Microcolony survival assay for cells of mouse intestinal mucosa exposed to radiation. Int J Radiat Biol Relat Stud Phys Chem Med 17: 261–267.
27. Ferraris RP, Villenas SA, Diamond J (1992) Regulation of brush-border enzyme activities and enterocyte migration rates in mouse small intestine. Am J Physiol 262: G1047–1059.
28. Eberts TJ, Sample RH, Glick MR, Ellis GH (1979) A simplified, colorimetric micromethod for xylose in serum or urine, with phloroglucinol. Clin Chem 25: 1440–1443.
29. Potten CS, Booth C (1997) The role of radiation-induced and spontaneous apoptosis in the homeostasis of the gastrointestinal epithelium: a brief review. Comp Biochem Physiol B Biochem Mol Biol 118: 473–478.
30. Kamata T, Katsube K, Michikawa M, Yamada M, Takada S, et al. (2004) R-spondin, a novel gene with thrombospondin type 1 domain, was expressed in the dorsal neural tube and affected in Wnts mutants. Biochim Biophys Acta 1676: 51–62.
31. Wei Q, Yokota C, Semenov MV, Doble B, Woodgett J, et al. (2007) R-spondin1 is a high affinity ligand for LRP6 and induces LRP6 phosphorylation and beta-catenin signaling. J Biol Chem 282: 15903–15911.
32. Binnerts ME, Kim KA, Bright JM, Patel SM, Tran K, et al. (2007) R-Spondin1 regulates Wnt signaling by inhibiting internalization of LRP6. Proc Natl Acad Sci U S A 104: 14700–14705.
33. Woodward WA, Chen MS, Behbod F, Alfaro MP, Buchholz TA, et al. (2007) WNT/beta-catenin mediates radiation resistance of mouse mammary progenitor cells. Proc Natl Acad Sci U S A 104: 618–623.
34. Hanson WR, Thomas C (1983) 16, 16-dimethyl prostaglandin E2 increases survival of murine intestinal stem cells when given before photon radiation. Radiat Res 96: 393–398.
35. Khan WB, Shui C, Ning S, Knox SJ (1997) Enhancement of murine intestinal stem cell survival after irradiation by keratinocyte growth factor. Radiat Res 148: 248–253.
36. Potten CS (1995) Interleukin-11 protects the clonogenic stem cells in murine small-intestinal crypts from impairment of their reproductive capacity by radiation. Int J Cancer 62: 356–361.
37. Potten CS, Booth D, Haley JD (1997) Pretreatment with transforming growth factor beta-3 protects small intestinal stem cells against radiation damage in vivo. Br J Cancer 75: 1454–1459.
38. Ch'ang HJ, Maj JG, Paris F, Xing HR, Zhang J, et al. (2005) ATM regulates target switching to escalating doses of radiation in the intestines. Nat Med 11: 484–490.
39. Paris F, Fuks Z, Kang A, Capodieci P, Juan G, et al. (2001) Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. Science 293: 293–297.

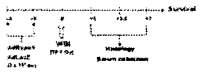



## Radiation Induced Gastrointestinal Injury (RIGS)




## Can administration of R-spondin1, an intestinal stem cell growth factor, protect against RIGS?

Experimental Design:

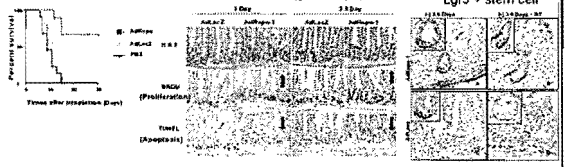


R-spondin-1 Treatment

Improves Survival

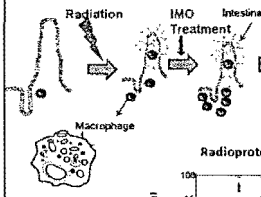
Increases Crypt cell proliferation
Decreases Crypt cell apoptosis

Increases number of Lgr5 + stem cell



## Does the addition of IMO, a TLR9 agonist, to R-spondin-1 increase the amount of radioprtoection against RIGS?

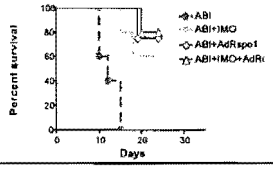
Hypothesis:



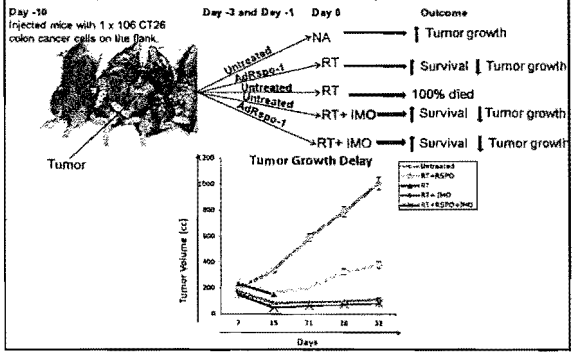

•Activate macrophages
•Increases Crypt cell proliferation
•Protects against sepsis

Increases overall survival

Radioprotection after ABI




## IMO and R-spondin-1 Ameliorate against RIGS. Does IMO and R-spondin-1 treatment also radioprotect the tumor?




## Discussion

•AdRspo1 and IMO treated group showed significant survival after 14Gy ABI compared to only irradiated ($p<0.0001$) cohort.

•AdRspo1 and IMO treatment did not delay tumor growth. There was significant delay in tumor growth and improved survival in AdRspo1 ± IMO-treated animals

•Significant delay in tumor growth was found in irradiated (14Gy ABI) groups compared to nonirradiated cohort.

•These results demonstrate that AdRspo1 combined with IMO could increase the therapeutic ratio of radiation therapy for the treatment of abdominal tumors where it would increase the tolerance of the intestine to irradiation without providing radioprotection to the tumor.

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# RADIOPROTECTIVE NEW CHEMICAL ENTITY
# ON 01210.Na (Ex-RAD™)

Onconova Therapeutics, Inc. is a private biopharmaceutical company located in Lawrenceville, NJ and Newtown, PA. Onconova is developing a new chemical entity, ON 01210.Na, as a radiation protection drug candidate for Acute Radiation Syndrome (ARS) in conjunction with the Armed Forces Radiation Research Institute (AFRRI) and the Department of Defense. The radioprotective drug is a first in class non-steroidal NCE with a novel mechanism of action. It is indicated for prophylactic use to enhance survival in personnel who are in imminent danger of exposure to life-threatening levels of x-ray or gamma radiation, and/or for therapeutic use to enhance survival in personnel who have received life-threatening levels of radiation. Ex-RAD™ is not a free-radical scavenger; its action is mediated by modulation of DNA repair pathways. Ex-RAD™ is protected by issued U.S. and foreign patents.

The prophylactic use of Ex-RAD™ has been demonstrated in two animal species. Additionally, the mitigating effect of the drug was recently demonstrated in the mouse whole body irradiation model, where two doses of drug were administered at **24** and **36** hours post-radiation. Onconova, with its collaborators, has demonstrated that the biological efficacy of Ex-RAD™ is mediated through enhanced DNA repair, protection of GI stem cells and the protection of progenitor cells in bone marrow.

## Current Status

The FDA-mandated GLP toxicology studies have been completed in two species. Scale up and manufacturing of Drug Substance and Drug Product have been accomplished under cGMP. Two Phase I human clinical trials have been completed under IND #67,526. A total of 52 healthy volunteers were involved in these trials. The drug is well absorbed following subcutaneous administration, with no evidence of systemic side effects in any of the volunteers.

Ex-RAD™ has high oral bioavailability and is safe to administer by a variety of routes and formulations. Extensive safety data from IV and SC toxicology studies in rodents and canine models are available. The GLP toxicology of the oral form is in progress. The oral form of Ex-RAD™ is expected to enter human clinical trials in the first quarter of 2010.

## Ex-RAD™ in Radiotherapy

Radiotherapy of cancer is established as a primary treatment option for breast, prostate and CNS tumors. Combinations of chemotherapy with radiation and surgery with radiation are routinely employed. Thus far, the efficacy of radiation therapy is limited by the side effects caused by injury to normal tissue. Therefore, a radioprotective compound would be of great value.

Ex-RAD™ has been evaluated in several *in vitro* and *in vivo* models. *In vitro* studies were conducted with MCF-7 and H80 glioblastoma cell-lines. Cell growth curves after various doses of radiation were compared with or without treatment with Ex-RAD™. These studies revealed that Ex-RAD™ was not radioprotective for cancer-cell lines. Instead a radiosensitizing effect was evident. In a MCF-7 xenograft tumor model in mice, Ex-RAD™ significantly improved the efficacy of single dose beam radiation localized to the ectopically growing tumor. These studies strongly suggest the potential for use of Ex-RAD™ in the radiotherapy setting. Combined with the demonstrated protective effect on the hematopoietic and gastrointestinal tissues, the ability to enhance radiation mediated apoptosis in cancer cells provides a compelling rationale for further exploration, including in the clinic.

Preliminary efficacy of Ex-RAD™ in the models of Radiotherapy coupled with a clean safety profile in humans provides a compelling justification for testing its effectiveness in cancer patients. Ex-RAD™ is under an IND and cGMP drug is available for parenteral and oral administration.

## Key People from Onconova

**Ramesh Kumar, Ph.D.:** Dr. Kumar is a co-founder of Onconova and serves as President and CEO. He received his Ph.D. in Molecular Biology from the University of Illinois, Chicago, and trained at the National Cancer Institute. He has held positions in R&D or management at Princeton University, Bristol-Myers Squibb, DNX (later Nextran, a subsidiary of Baxter) and Kimeragen (later Valigen), where he was President of the Genomics and Transgenics Division. Dr. Kumar has more than 50 publications spanning the areas of molecular oncology, transgenic animals, gene therapy and recombination.

**Manoj Maniar, Ph.D.:** Dr. Maniar received his B.S. in Pharmacy from Bombay College and his Ph.D. in Pharmaceutics from the University of Connecticut. He has led the development and commercialization of several products and medical devices over the past 20 years. Prior to joining Onconova, Dr. Maniar was with SRI International, where he served as Senior Director, Formulations and Drug Delivery. He has authored more than 100 patents, publications, and presentations in the field of pharmaceutical sciences. Dr. Maniar is the Senior Vice President of Development and heads the Ex-RAD™ program

**Charles Poryzees:** Mr. Poryzees is trained as a chemist with a B.S. and M.A from West Chester University. He has extensive experience as an IT professional and is the Project Manager for the Ex-RAD™ program.

**Francois Wilhelm, M.D, Ph.D.**: Dr. Wilhelm is Board Certified in Rheumatology, receiving his medical degree from Paris University Medical School, his Ph.D. in Endocrinology and a Master's degree in Biostatistics, both from the University of Paris. He has 23 years of clinical development experience covering all phases of drug development and post-marketing in Europe and in the U.S. He has been involved in clinical development programs in many therapeutic areas and has authored more than 30 publications.

## Key Collaborators

**E. Premkumar Reddy, Ph.D.**: Dr. Premkumar Reddy is a renowned scientist with a specific interest in molecular oncology. He is Director of the Fels Institute for Cancer Research and Molecular Biology at Temple University. He is the author of more than 200 publications and inventor on several dozen patents and applications. He founded Onconova Therapeutics in 1998. He was co-editor of the journal of Oncogene, published by Nature Publishing Group for more than 15 years. Among Dr. Reddy's many accomplishments are the co-invention of a diagnostic procedure used in HIV AIDS testing and the novel drug candidates being developed by Onconova.

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**John L. McManus, President & CEO**

# Advanced Development of AEOL 10150 as a Medical Countermeasure for Acute Radiation Syndrome and Cancer Radiation Therapy

AEOL 10150 is a broad-spectrum catalytic antioxidant specifically designed to neutralize reactive oxygen and nitrogen species. The neutralization of these species reduces oxidative stress, inflammation, and subsequent tissue damage-signaling cascades related to these events can induce.

| AEOL10150 Overview | |
|---|---|
| *Product Type* | Catalytic antioxidants (manganoporphyrin) |
| *Administration Route* | Subcutaneous administration; self-injection possible |
| *Indications in Development* | • Adjunct to radiation therapy<br>• Pulmonary ARS/DEARE<br>• GI ARS/DEARE<br>• Hematopoietic ARS/DEARE |
| *TRL Level* | TRL7/8 for Pulmonary Effects of ARS/DEARE |
| *Regulatory Status* | Active IND (IND-67741) |
| *Clinical Status* | Phase I (2 studies, 50 patients total 37 treated, 13 placebo) |

AEOL 10150 is currently in development for use as both a therapeutic and prophylactic drug in cancer patients, and is currently at Technical Readiness Level (TRL) 7 as a MCM for the pulmonary effects of ARS and DEARE. Aeolus has an active Investigational New Drug Application (IND) on file with the US FDA for AEOL 10150 as a potential treatment for amyotrophic lateral sclerosis (ALS). Extensive toxicology and pharmacology packages are in place, and Aeolus has completed two Phase 1 safety studies in 50 humans demonstrating the drug to be safe and well tolerated. Chemistry, Manufacturing, and Controls (CMC) work has been completed, and pilot lots have been prepared for scaling up. Efficacy has been demonstrated in both ARS and DEARE in rodent studies, with AEOL 10150 treated groups showing significantly reduced weight loss, inflammation, oxidative stress, lung damage, and, most important, mortality. In these studies, AEOL 10150 also reduced the incidence and severity of pneumonitis and fibrosis. Therapeutic efficacy has been demonstrated up to 24 hours after exposure to ionizing radiation.

To evaluate AEOL 10150's ability to mitigate acute radiation-induced lung injury, mice were exposed to 15 Gy of upper half body irradiation (UHBI) and subsequently treated with AEOL 10150. Animals received treatments subcutaneously beginning 2 hours after irradiation (20 and 40 mg/kg initial loading dose, respectively) followed by a maintenance dose of half the initial dose three times per week for 4 weeks. Results demonstrate that treatment with AEOL 10150 increased survival, maintained body weight, protected lung tissue, and reduced oxidative stress (via DNA and protein oxidation).

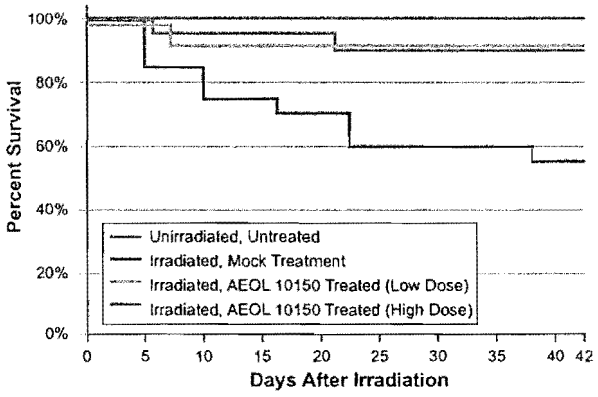


**Figure 1. Kaplan Meier survival curves for C57BL/6J mice after upper half body irradiation.** *The survival data displayed that there were no deaths in the sham-irradiated animals and animals receiving drug alone. In contrast, 9/20 (45 percent) of the animals that received 15 Gy UHBI died during the 6-week follow-up period. Treatment with low/high doses of AEOL 10150 markedly reduced radiation-induced mortality to only 10 percent (2/20).*

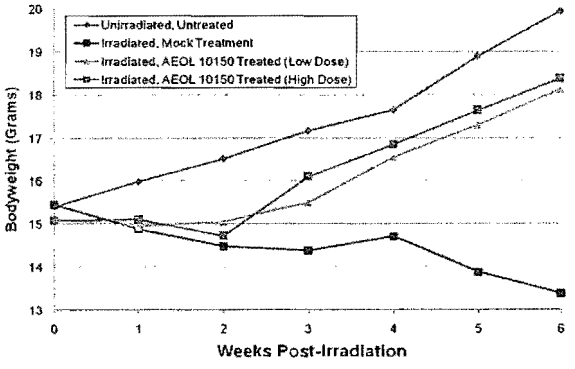


**Figure 2: Average body weight changes among groups.** *UHBI alone mice demonstrated significant weight loss beginning 3 weeks post-exposure compared with UHBI + low/high doses of AEOL10150 groups. Note that all animals in the Irradiated, Mock Treatment group had to be sacrificed due to weight loss at 42d post-irradiation. Of animals from the treated groups (6 total, 3 low dose, 3 high dose) retained after 42 days, 5 (3 high dose, 2 low dose) demonstrated survival until at least 6 months post-irradiation (data not shown).*

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# AEOL 10150 Development Program - Key Personnel

## Thomas MacVittie, Ph.D., Professor, University of Maryland

**Education and Training**

| Institution and Location | Degree | Year(s) | Field of Study |
|---|---|---|---|
| Biology, Alfred University, Alfred, NY | B.S. | 1964 | Biology |
| State University of New York, Buffalo, NY | M.S. | 1966 | Radiation Biophysics |
| State University of New York, Buffalo, NY | Ph.D. | 1970 | Radiation Biology |

**Positions**

| | |
|---|---|
| 1994-2000 | Radiobiologist, United States Army Medical Research Laboratory, Ft. Knox, KY. |
| 1972-1976 | Research Physiologist, Armed Forces Radiobiology Research Inst. (AFRRI), Bethesda,MD |
| 1976-1982 | Chief Hematology Division, Experimental Hematology Department, AFRRI, Bethesda,MD |
| 1982-1995 | Chairman & Project Leader, Experimental Hematology Department, AFRRI, Bethesda,MD |
| 1995-Current | Professor, UMD, Greenebaum Cancer Center, Dept.of Rad Onc & Pathology, Baltimore,MD |

**Other Experience and Professional Memberships**

| | |
|---|---|
| 1992, 1994 | WHO: Scientific Advisor, 4th (Ulm, Germany, 1992) & 5th (Paris, France 1994) Coordination Meetings of WHO Collaborating Centers in Radiation Emergency Medical Preparedness and Assistance |
| 1995-2002 | Board Member: International Association of Radiopathology, Fontenay-Aux-Roses, France. |
| 1998-2000 | Editorial Board, Journal of Experimental Hematology |
| 2001-Present | Editorial Board, The Journal of Stem Cells |
| 2003-Present | Member, CDC, Strategic National Stockpile Radiation Working Group |
| 2004-Present | NIH, NIAID, Special Emphasis Panel: Cooperative Research for the Development of Vaccines, Adjuvants, Therapeutics and Diagnostics for Biodefense and SARS. |
| 2005 | Ad Hoc Review, Chairperson, Scientific Review Program, "Myeloid Progenitor Cell Therapy for Radiation Exposure" |
| 2006-Present | Administered Ad Hoc Review Committees: NIAID, DTRA |
| 2006-2009 | Member ICRP Task Group: Radiation Effect on Normal Tissues |
| 2007 | Consultant, Co-Chairperson: IAEA Radiological Studies of Normal Tissue Effects in the 1-10 Gy Range and Higher, Relevant to Nuclear Accidents and other Radiation Incidents |
| 2007-2010 | Board Member, National Biodefense Science Board |

**Peer-Reviewed Publications (Selection from 141 peer-reviews publications)**


1. Farese, A.M., Hunt P., Boone, T., MacVittie, T.J. (1995) Recombinant human megakaryocyte growth and development factor stimulates thrombocytopoiesis in normal primates. Blood 86:54-59.
2. Farese, A. M., Hunt, P., Grab, L. B., MacVittie, T. J. (1996) Enhancement of hematopoietic reconstitution in nonhuman primates following radiation-induced marrow aplasia by the combined administration of recombinant human megakaryocyte growth and development factor and granulocyte colony stimulating factor. J Clin Inves 97:2145-215
3. Rosenzweig, M., MacVittie, T.J., Harper, D., Hempel, D., Glickman, R. L., Johnson, R. P., Farese, A.M., Whiting-Theobald, N., Linton, G.F., Yamasaki, G., Jordan, C.T., Malech, H.L. (1999) Efficient and durable gene marking of hematopoietic progenitor cells in nonhuman primates. Blood 94:2271-2286.


## Zeljko Vujaskovic, M.D., Ph.D, Duke University

**Education and Training**

| Institution and Location | Degree | Year(s) | Field of Study |
|---|---|---|---|
| Univ. of Zagreb Medical School, Zagreb, Croatia | M.D. | 1985 | Medicine |
| Colorado State University, Fort Collins, CO | Ph.D. | 1994 | Radiation Biology |

**Positions**

| | |
|---|---|
| 1985-1986 | Internship, Medical Centre Karlovac, Croatia |
| 1986-1987 | Residency, Military Medical Academy, Belgrade, Yugoslavia, and Rijeka, Croatia |
| 1987-1989 | Residency, Medical Centre Karlovac, Croatia |
| 1989-1990 | Fellow, Division of Medical Oncology, University of Colorado Cancer Center, Denver, CO |
| 1990-1994 | Post-Doctoral Fellow, Dept. of Radiological Health Sciences, Colorado State University, Fort Collins, CO |
| 1994-1998 | Assistant Professor, Dept. of Radiotherapy and Clinical Radiobiology, University of Groningen School of Medicine Groningen, Netherlands |
| 1999-2002 | Professor, Dept. of Radiation Oncology, Duke University of Medical Center, Durham, NC |
| 2004-2008 | Assoc. Director, General Clinical Research Center, Duke University Medical Center, Durham, NC |
| 2002-Current | Assoc. Prof., Dept. of Radiation Oncology, Duke University Medical Center, Durham,NC |

**Peer-Reviewed Publications (Selection from 80 peer-reviews publications)**


1. Gauter-Fleckenstein, B.; Fleckenstein, K.; Owzar, K.; Jiang, C.; Batinic-Haberle, I.; Vujaskovic, Z. Comparison of two Mn porphyrin-based mimics of superoxide dismutase in pulmonary radioprotection. Free radical biology & medicine 44:982-989; 2008.
2. Ghafoori, P.; Marks, L. B.; Vujaskovic, Z.; Kelsey, C. R. Radiation-induced lung injury. Assessment, management, and prevention. Oncology (Williston Park, N.Y 22:37-47; discussion 52-33; 2008.
3. Sugahara, T.; van der Zee, J.; Kampinga, H. H.; Vujaskovic, Z.; Kondo, M.; Ohnishi, T.; Li, G.; Park, H. J.; Leeper, D. B.; Ostapenko, V.; Repasky, E. A.; Watanabe, M.; Song, C. W. Kadota Fund International Forum 2004. Application of thermal stress for the improvement of health, 15-18 June 2004, Awaji Yumebutai International Conference Center, Awaji Island, Hyogo, Japan. Final report. Int J Hyperthermia 24:123-140; 2008.

## Innate Defense Regulator drug: Mitigation of Acute Radiation Injury

## Inimex Pharmaceuticals, Inc.

**Summary:**

Inimex Pharmaceuticals, Inc. is developing a new class of agents, Innate Defense Regulators (IDRs). Designed to mimic one of the functions of natural mucosal defense peptides, IDRs protect against – and treat - infections by selectively modifying the responsiveness of the body's innate defenses, without triggering inflammatory responses. IMX942, the first IDR drug to enter formal development has completed phase 1 safety trials in healthy volunteers and reduces infection and inflammation in animal models. IMX942 is effective in animals rendered neutropenic by chemotherapy and data from a preliminary study in a mouse radiation model suggests the possibility that IMX942 may reduce radiation-induced mucosal organ damage and infection.

**Key personnel to attend the ART-RIM Meeting:**

**John R. North, Ph.D., Chief Operating Officer, Inimex Pharmaceuticals, Inc.**
Dr. North has been leading R&D teams within the biotechnology / pharmaceutical industry for over 25 years. He led much of the development of Inimex' IDR technology, serving as Executive Vice-President of R&D in 2004 and then President and Chief Executive Officer 2005 to 2009. Prior to joining Inimex, he served as Sr. Vice President, Scientific Affairs and Chief Scientific Officer of QLT, Inc., Vancouver, BC, Canada. Earlier in his career, Dr. North was Managing Director of Monotech Laboratories Ltd., a biotech start-up in UK developing monoclonal antibodies, and was then a Biotechnology Consultant at PA Technology Ltd., Cambridge, UK. For 12 years he served in various R&D capacities within UK- and US-based subsidiaries of the Beaufour Ipsen Group, most recently as Head of Exploratory Development at Ipsen International in the U.K.

Dr. North received a Ph.D. in Immunology and an M.A. in Natural Sciences from the University of Cambridge, England. He subsequently completed post-doctoral studies at the MRC National Institute for Medical Research, London, at UC Berkeley and at the Salk Institute, San Diego before leading a team at Bristol University in UK.

**Contact:** jnorth@inimexpharma.com Cell: 604 230 3501 www.inimexpharmaceuticals.com

**About IMX942:**

A placebo-controlled phase 1 study in healthy volunteers has been completed and demonstrated that single intravenous doses of IMX942 were well tolerated up to the maximum tested. Daily intravenous doses were also well tolerated (7 daily doses).

IMX942 is a proprietary, highly water-soluble, synthetic, 5 L-amino-acid peptide. IMX942 has been designed as an intravenous drug for administration in the hospital context and would therefore be suitable for use in management of ARS. Intravenous IMX942 is currently under development by Inimex for the prevention and treatment of recurrent infections and infections in hospitalized patients at high risk of infection, such as those in intensive care or undergoing high dose chemotherapy.

The innate immune response is the first line of defense against infectious agents, closely associated with mucosal and epithelial barrier functions – systems that show early pathology in Acute Radiation Syndrome (ARS). IMX942 binds to an intracellular adaptor protein, Sequestosome-1 (SQSTM-1), also known as p62, that is involved in the efficient transmission of information during intracellular signal transduction, receptor trafficking, protein turnover and bacterial clearance. p62 has recently been shown to function at a key nodal position in this signalling network, interacting with key kinases and ligases downstream of TLR and Tumor Necrosis Factor (TNF) receptors. IMX942 binding to SQSTM-1 selectively alters its interactions with other proteins in these critical signalling cascades. Unlike drugs targeting the TLRs themselves, the binding of IMX942 does not cause persistent activation of NFκB, the central transcription factor associated with potentially harmful inflammatory responses. Production of pro-inflammatory cytokines such as TNF-α in response to injury and pathogen challenge is suppressed by IMX942 treatment while the transcription factor C/EBPβ is activated to increase expression of chemokines, including MCP-1. In vivo studies show that IMX942 promotes monocyte and macrophage recruitment to disease sites and speeds resolution of disease.

IMX942 has no direct antibacterial activity. IMX942 can selectively up-regulate innate defences within hours, while controlling the attendant inflammatory response. The pharmacodynamic action of a single dose of IMX942 is prolonged (2-3 days), even though clearance of the drug from circulation is rapid. Moreover, studies in animal infection models have shown that a single dose of IMX942, when given with a sub-optimal level of antibiotics, increases the survival of infected animals. IDRs target the host response (not the specific pathogen) – and therefore act on the pathophysiology caused by a broad spectrum of agents, whether antibiotic resistant or not, No hyperactivation or suppression of adaptive immune responses, or other impact on the phenotypes of cells associated with adaptive immunity, has been detected following IMX942 administration.

In a preliminary study, administration of IMX942 to mice after exposure to 6.5 Gy from a $^{137}Cs$ source reduced the number of clinical and gross pathological observations, particularly those in exocrine organs, skin and subcutaneous tissues. Survival was prolonged by approximately 1 week. These data suggest that IMX942 may have the potential to complement other treatments to mitigate sub-syndromes associated with ARS. Further studies are planned.

A number of studies have also been conducted in neutropenic mouse infection models, where neutropenia is induced by chemotherapeutic (cyclophosphamide) administration. As expected, IMX942 did not alter the recovery of circulating blood cell counts. However, IMX942 - either alone or in combination with antibiotics - was clearly beneficial in resolution of an infectious challenge. This data suggests that IMX942 will likely be broadly effective against infections in neutropenic individuals, including those affected by lethal radiation.

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XOMA

**A proposal to mitigate inflammatory complications and enhance recovery from the effects of acute post-radiation injury**

XOMA, a fully integrated biotechnology drug discovery and development company based in Berkeley, California, is focused on developing innovative monoclonal antibody therapies for the treatment of diabetes and other cardiometabolic diseases, inflammatory disorders, oncology, and infectious diseases. XOMA has a staff of approximately 200, representing the diverse skill base, expertise, and experience necessary for drug discovery and development. Founded in 1981 by Dr. Patrick Scannon, XOMA has been a long-standing, consistent innovator in monoclonal antibodies, antibody-drug conjugates, and protein therapeutics discovery and development. XOMA has worked in all of the major therapeutic areas including inflammation, autoimmunity, transplantation, oncology, and infectious diseases.

As a drug development company, XOMA's extensive knowledge and expertise in the antibody field supports the discovery, development, and manufacture of its own proprietary pipeline as well as with premier pharmaceutical partners, such as Novartis AG, Chiron, Merck & Co., Inc., Schering Plough, and Takeda, and world-renowned research institutions, including the Dana-Farber Cancer Institute. XOMA also has a 4 year history of working with the US government through the National Institute of Allergy and Infectious Diseases (NIAID) on multiple therapeutic projects, including development of a panel of anti-botulinum antibodies, SARS antibodies, and influenza (H1N1 and H5N1) antibodies. XOMA's technologies have contributed to the success of marketed antibody products, including LUCENTIS® (ranibizumab injection) for wet age-related macular degeneration and CIMZIA® (certolizumab pegol) for rheumatoid arthritis and Crohn's disease.

With 29 years of experience, XOMA's premier antibody discovery and development platform incorporates our industry leading collection of antibody phage display libraries, including libraries developed by XOMA, and proprietary Human Engineering™, affinity maturation, Bacterial Cell Expression (BCE) and novel manufacturing technologies. XOMA's patents are licensed to over 75 companies. XOMA has extensive experience in the conduct of clinical trials under Good Clinical Practices at every stage of development with particular emphasis on mid stage clinical trials to identify safe and efficacious doses of different biologic drugs in multiple disease states. XOMA's medical personnel have an average of over 25 years of experience conducting clinical trials.

With over two decades of working with US and international regulatory authorities, XOMA has extensive regulatory experience in bringing biotherapeutics into the clinic in the US and internationally. XOMA's regulatory experience expands from first-in-human and early clinical development through conduct of large phase 3 clinical trials covering over 25 acute and chronic disease indications, both as an independent company and in collaboration with other companies.

XOMA's oncology expertise

Related to oncologic diseases, XOMA's selection process takes into account existent literature, existent intellectual property, and specific XOMA expertise toward designing novel products. XOMA and its staff have been investigating oncologic diseases for its entire 29 year history, developing a number of novel antibody therapeutic approaches. XOMA was originally founded on the promise of using monoclonal antibodies to direct toxins to tumor cells and to neutralize endogenous pathogens involved in disease processes. Throughout XOMA's history, products were developed and evaluated in clinical testing, which included naked antibodies in hematological malignancies; colorectal, pancreatic and gastric cancers; immunoconjugates specific for malignant melanoma; colorectal and breast cancer; and antibodies for the treatment of graft-versus-host disease following allogeneic bone marrow transplantation.

Currently, XOMA is assessing several antibody product configurations taking advantage of XOMA's unique technology base. Depending on the tumor targets, XOMA is considering incorporating into its oncology portfolio: ultra-high affinity antibodies, cocktails of antibodies to different epitopes or antigens and antibody-drug conjugates. Details about XOMA's Preclinical Oncology Program are available for further discussion.

**XOMA 052, a high-affinity binding anti-interleukin-1β monoclonal antibody therapeutic candidate, is proposed for study to mitigate inflammatory complications and enhance recovery from the effects of acute post-radiation injury**

Infectious complications are enhanced in ARS by radiation-induced epithelial cell death resulting, for example, in disruption of the lining of the gastrointestinal (GI) tract. This combination of neutropenia and GI disruption enhances systemic invasion of microbes and endotoxin into the affected human. A critical constitutively-produced human defense protein found within mature neutrophils and epithelial cells is bactericidal permeability increasing protein (BPI), and an important consequence in ARS patients with neutropenia and GI epithelial cell death is the quantitative reduction of available BPI to fight infections. Initially, XOMA and the Dana Farber Cancer Institute has extensively studied Opebacan, (recombinant 21kd fragment of BPI, Neuprex®), *in vitro* and *in vivo* in both animals and humans. Endotoxin-induced production of TNF-α, nitric oxide, free radicals, E-selectin and CD54 was attenuated or reversed in the presence of $rBPI_{21}$.

This finding coupled with XOMA's development of a next-generation highly potent anti-inflammatory monoclonal antibody leads XOMA to believe that a therapy that could mitigate the inflammatory complications and enhance recovery after exposure to radiation injury would be a valuable adjunct to existing therapies. This next-generation anti-inflammatory monoclonal antibody would exhibit desirable properties, such as high binding/highly efficacious, long half life (to suggest monthly dosing), a clean safety profile, and intramuscular or subcutaneous delivery options.

Inflammation resulting from radiation induces massive cytokine release. At the core of the cytokine pathway is the IL-1 family of mediators. IL-1β is a key pro-inflammatory mediator that is central to many of the pathologic aspects of acute and sub-acute radiation damage: lung and tissue fibrosis; disruption of bone marrow stromal integrity leading to poor engraftment of hematopoietic stem cells; and initiation of pyrogen-induced cytokine release, as occurs after breakdown of the intestinal barriers, which can lead to systemic inflammatory syndrome associated with bone marrow failure and perpetuation of sepsis-like syndrome.

XOMA 052 is a Human Engineered™ monoclonal antibody (mAb) that binds human IL-1β with 0.3 pM affinity and regulates the activation of IL-1 receptors. XOMA 052 is currently in Phase 2 clinical trials. Administration of XOMA 052 to patients suffering from IL-1β-mediated systemic inflammatory diseases is expected to produce rapid and sustained reductions in symptoms. Support for the ability of XOMA 052 to inhibit inflammation comes from various cell-based functional assays. XOMA 052 was shown to inhibit IL-1β-mediated IL-6 expression from the human fibroblast cell line MRC5. In a second assay, XOMA 052 inhibited IL-1β-mediated IL-8 expression from human whole blood. In this assay, the IC50 for XOMA 052 was 28 ± 18 pM, still significantly more potent than anakinra (608 ± 295 pM). Human whole blood cultures evaluating toll-like receptor (TLR) agonist stimulation also indicate that XOMA 052 inhibited the production of IL-1β by 50% at 0.1 pM. Significant effects on the production and/or release of IL-1α, IFNγ, TNFα, and IL-6, but not IL-1Ra, also were seen.

In phase 1 clinical trials, following 6 months of monthly treatment with XOMA 052, T2D patients had a reduction in markers of inflammation, CRP, IL-6, and IL-8, and a decrease in blood pressure, and PAI-1, VCAM and E-selectin, levels. XOMA 052 has demonstrated an extraordinarily clean safety profile in humans; also throughout the entire non-clinical toxicology program, no drug-related safety findings of any kind have been observed. XOMA 052 is a Human Engineered™ antibody with a long half-life after dosing and ultra-high affinity for IL-1β, leading to convenient dosing of once per month or longer, rather than taking oral medications one or more times per day.

IL-1β is a growth stimulus for many known tumors, including lung cancer, melanomas and multiple myeloma. This potential study would contribute to the ongoing work for IL-1 blockade as an adjuvant therapy for these tumors. Based on XOMA's preclinical and clinical data and supporting literature, XOMA 052 may modulate the immune system leading to decreased time to death and/or a decrease in inflammatory markers. Results of XOMA 052 in human studies to date have shown that the antibody is safe and show a reduction in inflammatory markers.

**Xoma (US) LLC**
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**Berkeley, CA 94710**
**Ph: 510- 926-5933**
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Key Personnel

**Gere diZerega, MD**

US Biotest, Inc of Southern California KECK School of Medicine Professor

**Kathleen Rodgers, Ph.D**

US Biotest, Inc. Scientific Director

University of Southern California KECK School of Medicine Professor

**Rick Franklin, MD, Ph.D.**

Tarix CEO

**Parvesh Kumar, MD**

University of Southern California, KECK School of Medicine, Chair Radiation Oncology

Product

Epithelial tissues, including oral mucosa and skin, can sustain radiation damage. Epithelial radiation injury can result in lesions from direct exposure or indirectly by damage to progenitors that would otherwise contribute to healing. Radiation injury can also occur during clinical exposure, particularly during x-ray guided intervention and in sensitive populations. In addition, the majority of patients undergoing extended field intensity modulated radiotherapy (EF-IMRT) for head and neck cancer experience Stage 2 erythema, dermatiis or worse often requiring topical therapy for this side effect. Further, the vast majority of patients undergoing radiotherapy for head and neck cancer experience severe stomatitis. In some individuals, radiation induced dermatitis and/or stomatitis necessitates delay in therapy and the associated reduction in efficacy. The effectiveness of angiotensin peptides to stimulate epithelial healing after dermal injury or chemotherapy has been shown in preclinical and clinical studies.

US Biotest, Inc. has shown that angiotensin peptides can rapidly promote epithelial healing and hematopoiesis, both of which may be needed to heal burns that occur during radiation blasts. We have found in pre-clinical studies that angiotensin II (AII) and angiotensin (1-7) (A(1-7)) promote tissue regeneration in animal models more quickly and effectively than comparative treatments. More recently, an analogue of A(1-7), $NorLeu^3$-A(1-7) was identified. In a series of *in vivo* studies in different animal models of wound repair, $NorLeu^3$-A(1-7) was superior in wound healing to AII, A(1-7) and the only FDA approved drug to increase wound healing, Regranex™.

We are proposing to study the effect of A(1-7) on radiation induced stomatitis after systemic exposure and the effect of $NorLeu^3$-A(1-7) on radiation induced dermatitis after topical application in patients undergoing radiotherapy for the treatment of head and neck cancer.

# GUIDANCE

# FOR

# INDUSTRY

# Guidance for Industry

## Patient-Reported Outcome Measures: Use in Medical Product Development to Support Labeling Claims

http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM193282.pdf

**U.S. Department of Health and Human Services**
**Food and Drug Administration**
**Center for Drug Evaluation and Research (CDER)**
**Center for Biologics Evaluation and Research (CBER)**
**Center for Devices and Radiological Health (CDRH)**

**December 2009**
**Clinical/Medical**

# ATTENDEES

# Advanced Radiation Therapeutics (ART) Radiation Injury Mitigation (RIM) Workshop

Co-Sponsored by NCI and NIAID Jan. 25, 2010

## *Incomplete* List of Participants

| | |
|---|---|
| Ben Movsas, M.D., Co-Chair<br>Chairman, Radiation Oncology<br>Henry Ford Health System<br>Herndon Chair in Oncology Research<br>Henry Ford Hospital<br>2799 West Grand Boulevard<br>Detroit, MI, 48202<br>Email: BMOVSAS1@hfhs.org | Paul Okunieff, M.D., Co-Chair<br>Director, Shands Cancer Center<br>Chairman, College of Medicine<br>Department of Radiation Oncology<br>University of Florida<br>2033 Mowry Road, RM 145<br>PO Box 103633<br>Gainesville, FL 32610-3633<br>Email: pokunieff@ufl.edu |
| Mitchell S. Anscher, M.D.<br>Professor and Chair Dept. of Radiation Oncology<br>Virginia Commonwealth Medical Center<br>401 College St<br>PO Box 980058<br>Richmond, VA 23298-0058<br>Email: manscher@mcvh-vcu.edu | Ethan M. Basch, M.D.<br>Memorial Sloan-Kettering Cancer Center<br>1275 York Avenue<br>New York, NY 10065<br>Tel. 212-639-2000<br>Email: basche@MSKCC.ORG |
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| Weiwen Deng, M.D., Ph.D.<br>Research Director<br>Pediatric Blood & Bone Marrow<br>Transplantation Program, MC185<br>Helen DeVos Children's Hospital<br>Spectrum Health<br>100 Michigan Street, NE<br>Grand Rapids, MI 49503<br>Ph: 616.233.8647 Fax 616.391.9233<br>Email: weiwen.deng@devoschildrens.org | James A. Deye, Ph.D., NCI<br>deyej@mail.nih.gov |
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| George Georges, M.D.<br>Fred Hutchinson Cancer Research Center<br>1100 Fairview Ave. N. D1-100<br>P.O. Box 19024<br>Seattle, Washington 98109-1024<br>Ph: 206 667-6886<br>Fax: 206 667-6124<br>Email: ggeorges@fhcrc.org | Chandan Guha, MD, PhD<br>Professor and Vice Chairman<br>Dept of Radiation Oncology<br>Montefiore Medical Center<br>Albert Einstein College of Medicine<br>111 East 210 Street<br>Bronx, NY 10467<br>Ph: 718-920-2702<br>Fax: 718-231-5064<br>Email: cguha@montefiore.org |
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