

A Phase I Neoadjuvant Study of *In-situ* REIC/Dkk-3 Therapy
Followed by Prostatectomy in Patients with
High Risk Localized Prostate Cancer

Protocol #. 1001-1026

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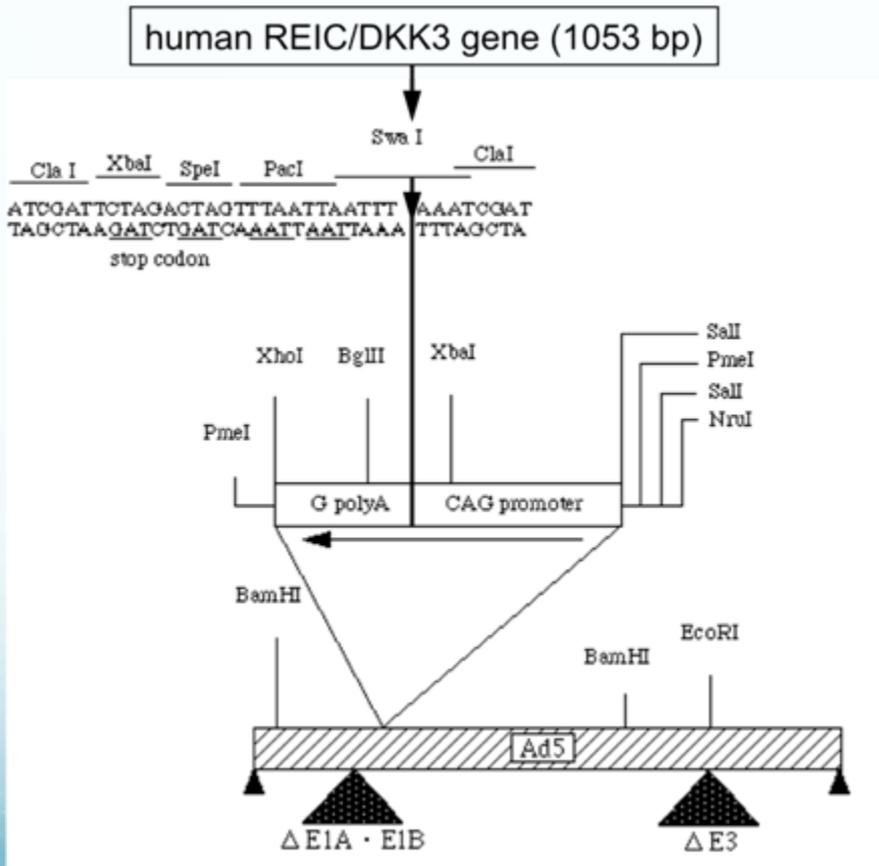
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REIC/Dkk-3 Gene

- Reduced Expression in Immortalized Cells (REIC)
- Deficient Many Types of Cancer Cells
- Member of the Dickkopf Gene Family (Dickkopf-3)
- Potential to Induce Apoptosis Through Activation of c-Jun-NH₂-kinase (JNK), c-Jun
- Mechanism of Apoptotic Cell Death Demonstrated to be Related to Endoplasmic Reticulum (ER) Stress
- Full Sequence Elucidated and Highly Homologous Among Mammalian Species

Ad-hREIC/Dkk-3

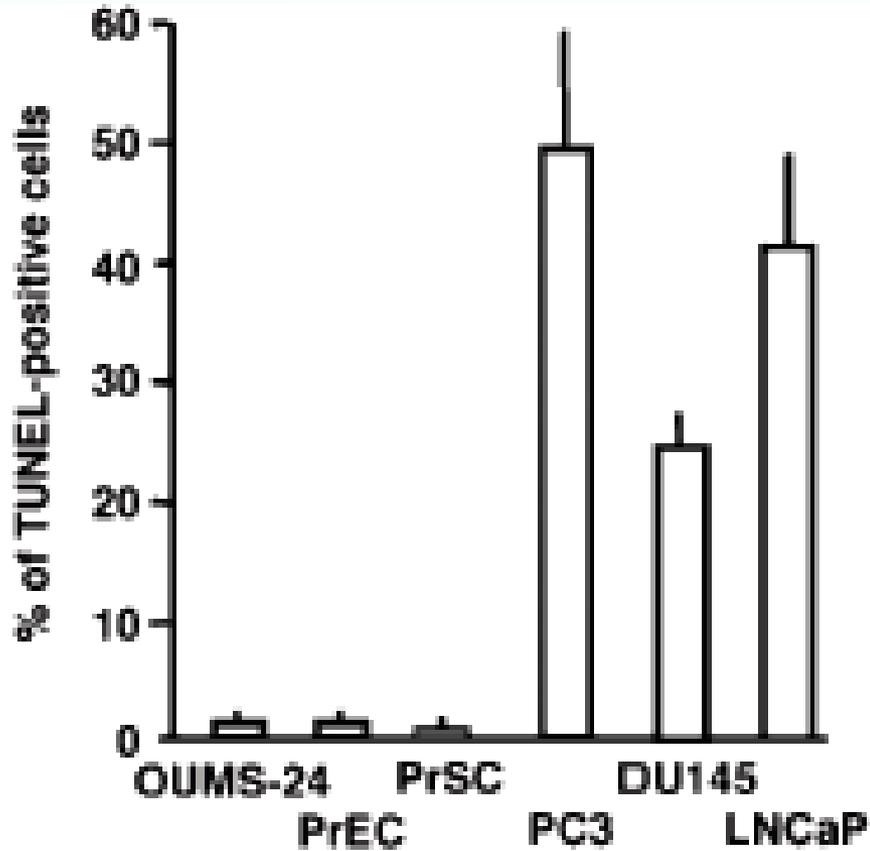
- Serotype-5 Adenovirus
- Replicate Incompetent
 - Deleted E1A, E1B, E3
- Insertion of Full Length REIC/Dkk-3 Gene
 - E1A, E1B Region
- CAG Expression of REIC: CMV-IE Enhancer and Bird B-actin promoter before the REIC Sequence and a Rabbit B-globulin polyA Tail



Ad-hREIC/Dkk-3

- Demonstrated to Induce Apoptosis in multiple Human Prostate Cancer Cells (PC3, DU145, LNCaP)
- Manufactured in GMP Viral Production Facility
- More than 20 Preclinical Experiments for Pharmacology and Efficacy
- Completed 4 Toxicology Studies in Relevant Species
 - NonGLP Tumor Bearing Mouse and normal Mouse
 - GLP Rat and Dog
 - No Significant Toxicity Observed at the Maximum Feasible Dose

Ad-hREIC/Dkk-3 *In-Vitro* Models



(Abarzua and Nasu 2005)

- Human REIC is Effective in Human Prostate Cancer Lines
 - PC3
 - DU145
 - LNCaP
- Human REIC is Effective in Mouse and Rat Prostate Cancer Lines
 - RM-9
 - YPEN1
- No Toxicity in 'Normal Prostate Cells'
 - Prostate Epithelial Cells (PrEc)
 - Prostate Stromal Cells (PrSc)
 - Fibroblast Cells (OUSM-24)

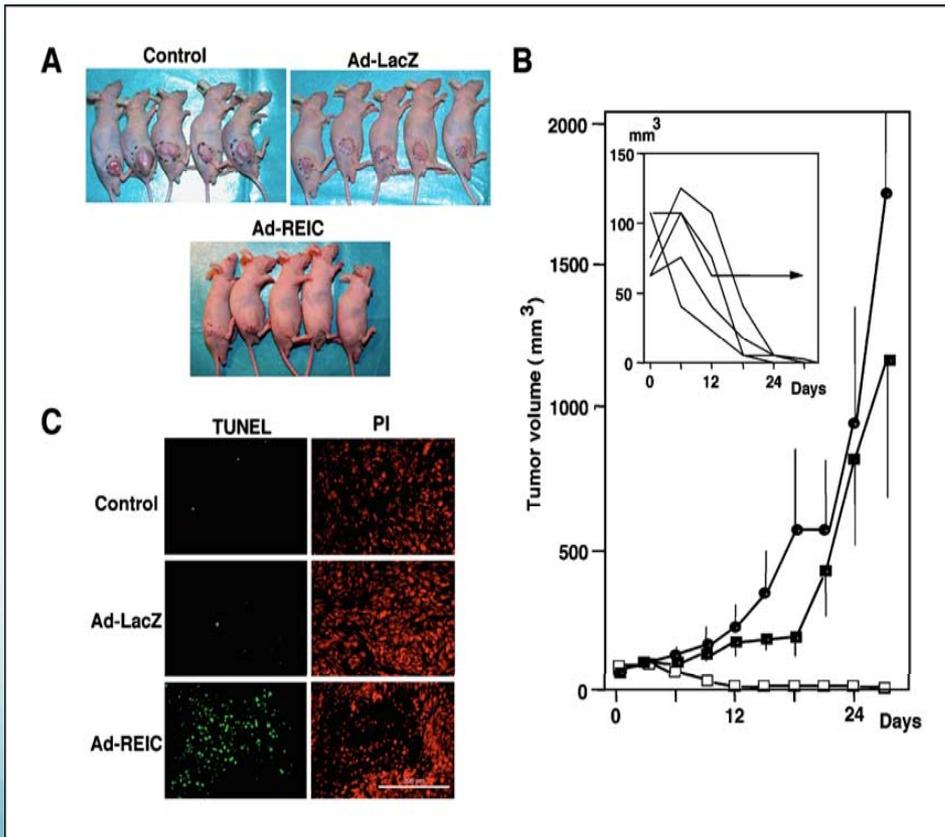
Immunological Aspects

- REIC/Dkk-3 Protein Induces the Differentiation of Human CD14+ Monocytes
 - Monocytes resemble immature dendritic cells
 - Similar affect to IL-4 and GM-CSF
- In vivo REIC/Dkk-3 Protein Significantly Suppressed Tumor Growth with CD11c+ and CD8+ (dendritic and killer T cell marker, respectively) cell accumulation
 - Enhances anti-cancer cytolytic activity of splenocytes
 - Suppressed pre-existing metastasis

(Watanabe and Nasu., Int.J.Oncol 2009)

Ad-hREIC/Dkk-3 *In-Vivo* Models

Human Xenograph Model

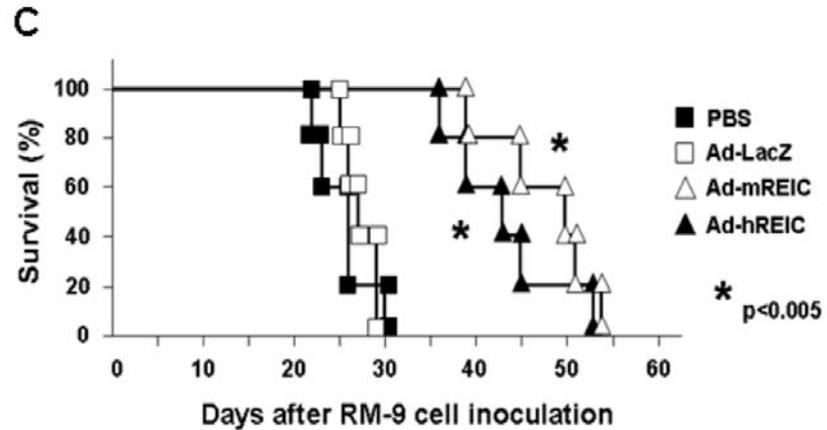
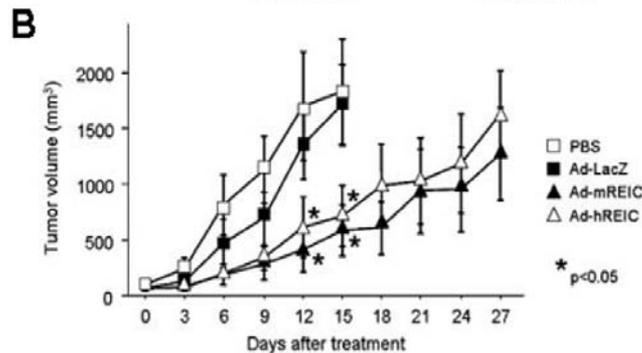
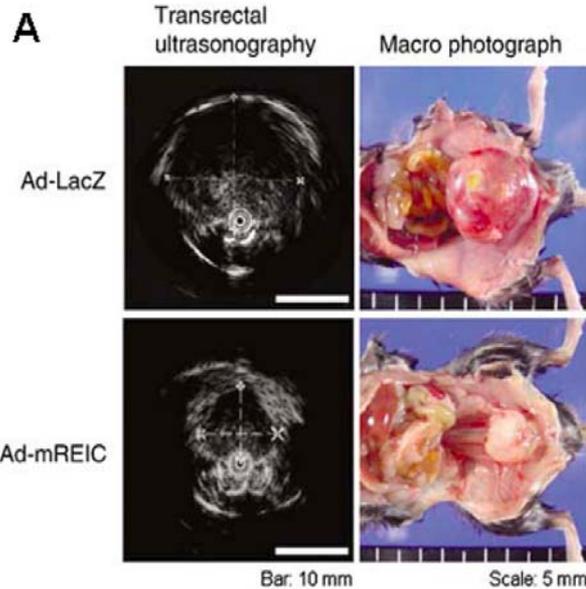


(Abarzua and Nasu 2005)

- Effective in Subcutaneous and Orthotopic Tumor Growth Suppression
- Suppression of Spontaneous Lymph Node Metastases
- Mouse and Human REIC Equally Effective
- No Significant Toxicity Observed at Efficacious Doses

Ad-hREIC/Dkk-3 In-Vivo Survival

Mouse Orthotopic Immunocompetent Model



A, Representative macroscopic and transrectal ultrasonographic views of orthotopic RM-9 tumors on day 15 after Ad-mREIC or Ad-LacZ treatment.

B, Orthotopic RM-9 tumors were formed and injected intratumorally with Ad-mREIC, Ad-hREIC, Ad-LacZ or PBS on treatment day 0. Tumor size was measured by TRUS; data represent the average of five individual mice in each group. Bars, standard error. A significant difference was observed (* $P < 0.05$) between the Ad-REIC and Ad-LacZ treatments.

C, Long-term survival of RM-9 tumor-bearing mice after intratumoral Ad-REIC delivery. Kaplan-Meier curve is shown in the Ad-mREIC-, Ad-hREIC-, Ad-LacZ- and PBS-treated groups, with each group consisting of five mice. There was a significant difference ($P < 0.005$) between Ad-REIC and Ad-LacZ treatments.

(Edamura and Nasu 2007)

Ad-hREIC/Dkk-3 Toxicology

- Four Toxicology Studies Performed with Ad-hREIC/Dkk-3 Injections Into the Prostate.
 - Tumor bearing mouse
 - Normal mouse
 - GLP Rat
 - GLP Dog
- Maximum Feasible Dose = NOAEL in both Rat (5×10^{10} vp) and Dog (1×10^{12} vp)
 - Mild inflammation in prostate and bladder
 - No other positive findings
- Margin of Safety (Human Starting Dose 1×10^{10} vp)
 - Rat: 1,550
 - Dog: 673

Ad-hREIC/Dkk-3 Summary

- Demonstrated to be Effective Against Human Prostate and Other Human Cancers
- Multiple Animal Efficacy Models in Human and Mouse Prostate Cancer
 - Efficacy Models Demonstrate Potential for Reduced Metastases
- Toxicology Studies in Relevant Species Demonstrate Low Potential for Toxicology
- Construct Based on Well Known Adenovirus Serotype-5 Construct (Safety Well Understood)
- Injection into the Prostate Planned for First-in-Man Studies in High Risk Prostate Cancer Patients

Protocol # 1001-1026

- Neoadjuvant Trial in High Risk Prostate Cancer Patients Scheduled to undergo a Radical Prostatectomy (T1c, T2, T3 and Gleason 7-10 or PSA \geq 10 ng/mL)
- Single-dose Injection into the Prostate
- Three Dose Levels – Dose Escalation Design
- Primary Assessment is Safety
- Secondary Assessments PSA / Tissue Histopathology
- Risk/Benefit: Prevention of Recurrence

Precedence

- Neoadjuvant Prostate Cancer Model in Patients Planned to Undergo a Radical Prostatectomy is Well Established
- NIH RAC Precedence with Adenovirus Products
 - OBA 0604-773: Ad5-RTVP-1
 - OBA 0309-603: Ad5-TRAIL
 - OBA 0010-428: Adenovirus Virus / Cytosine Deaminase
 - OBA 9909-229: Ad5-P16 cDNA
 - OBA 9801-229: Ad-HSV-Tk Gene Transfer
 - OBA 9710-217: Ad-INGN 201
- Main Entry Criteria for All Studies Deemed Acceptable
 - Gleason 7-10 or PSA \geq 10 ng/mL
- No reported problems with subsequent prostatectomy

Kattan Nomogram

- Widely Used in Japan, Less Well Established in US
- Original Publication JNCI 1998
- Recent Update BJUI Nov. 2009
 - Adjusted Risk Assessment
- Investigators Agree that Use of Kattan Nomogram as an Entry Criteria is Premature
- Recent Literature Review of Phase 1/2 studies in similar population Showed Limited Use of Kattan Nomogram

Conclusion of Investigators

- Neoadjuvant Prostate Cancer Model is an Appropriate Trial Design for First-in-Man Use of Ad-hREIC/Dkk-3
- Ad-hREIC/Dkk-3 has been Proven in Preclinical Models to be Effective Against Human Prostate Cancer and May Help Prevent Recurrence
- Entry Criteria Based on Gleason of 7-10 or PSA \geq 10 ng/mL is Appropriate and Considered Acceptable Risk Based on Past NIH RAC Precedence
- Low Risk to Patients Based on Relevant Animal Models
- High Potential for Significant Scientific and Medical Information to be Gained from the Proposed Trial Design

RAC Comments - Kahn

- Investigators Agree to Update the Patient Informed Consent to be more Clear that this Represents a First-in-Man use and Phase 1 Toxicity Study of Ad-hREIC/Dkk-3
- Investigators Agree to Modify Claims of Potential Benefit to Patients
- Barrier Contraceptives are Required by the Protocol for 4-weeks After Injection of Ad-hREIC/Dkk-3 prior to Prostatectomy. The Error in the Patient Informed Consent will be Corrected.

RAC Comments - Gulley

- Investigators will not use the Kattan Nomogram and Conform to Conventional Measures for High Risk Patients based on NIH RAC Precedence.
 - Entry Criteria will be Adjusted to Gleason 7-10 or PSA \geq 10 ng/mL
 - These Entry Criteria were Previously Considered by NIH RAC as an Acceptable Risk for This Patient Population.
 - Estimated BCR for this population approaches 50%
- Ad-hREIC/Dkk-3 has a Low Risk based on Toxicology Studies.
 - Safety Margins are Significant (Rat 1,550 Higher than Human Starting Dose)

RAC Comments – Gulley continued

- Neoadjuvant in-situ Gene Therapy Followed by Prostatectomy Using Adenoviral Vector
 - No Delay in surgery
 - Hall(2000) , Trachtenberg(2003),Bangma(2005),Ayala(2006),
- Experiences of Adenoviral Vectors in Prostate Cancer
 - Over 150 patients with Ad HSV-tk
 - Only one study with dose limiting toxicities
 - Most common toxicities: fever and chill
 - Another aprox 50 pts HSV-tk with Radiation (Replication comp. and incomp)
 - no fever and no chill

(Stanizzi and Hall 2007)

RAC Comments - Flint

- 1a) Main mode of action is Apoptosis
- 1b) Data has been presented today showing differential effects of REIC for human cancer and normal cells
- 2) Induction of Immunologic Response demonstrated
 - Data (slide 6) indicated a Cytokine-like Role of REIC/Dkk-3 Protein in Monocyte Differentiation
- 3) Effect of REIC over-expression in normal human cells
 - Data (slide 5) indicated in Normal prostate cells
 - Likewise data within investigator's brochures indicated in other normal human cells (SAEC,HUVEC,RPTEC,HC)

SAEC: small airway epithelial cell

HUVEC; human umbilical vein epithelial cell

RPTEC; renal prxismal tube epithelial cell

HC:hepatocyte cell

RAC Comments - Flint continued

- 4) Effects of REIC are Anticipated to be Acute
 - Clearance is within 7 days
 - Effect is within 2-3 days
- 5) The E1A, E1B and E3 Serotype-5 Adenovirus has been Demonstrated to have No Significant Potential to Replicate in Normal Tissue
 - Supported by *in-vitro* and *in-vivo* Preclinical Studies
- 6) Immune Response to Ad-hREIC/Dkk-3 will be Measured by WBC/Neutrophil, Cytokine Induction and Histopathology. No Other Specific Evaluations are Planned for this Phase 1 Protocol

RAC Comments - Buchmeier

- Investigators Believe that Risk in this Patient Population is Acceptable (see previous comments)
- Schedule for Prostatectomy is Not a Delay from Standard of Care (Typical 4-6 Weeks to Schedule)
- Prevalence of Ornithine transcarbamylase (OTC) is Considered to be Very Low in this Patient Population.
 - However, Investigators are open to evaluation if required by RAC
- The Adenovirus Serotype-5 Vector Used is Common
 - Investigators did Not Consider the Need for an Extensive Summary of Ad-5 Safety to RAC, but can Provide Review Articles or other Summaries as Needed.

Questions and Discussion