Protocol Title: Phase I Clinical Trial of Autologous Alpha-Folate Receptor Redirected T Cells Administered Intravenously in Ovarian Cancer Patients
RAC # 1010/1076

Presentation to the Recombinant DNA Advisory Committee
December 8, 2010

by

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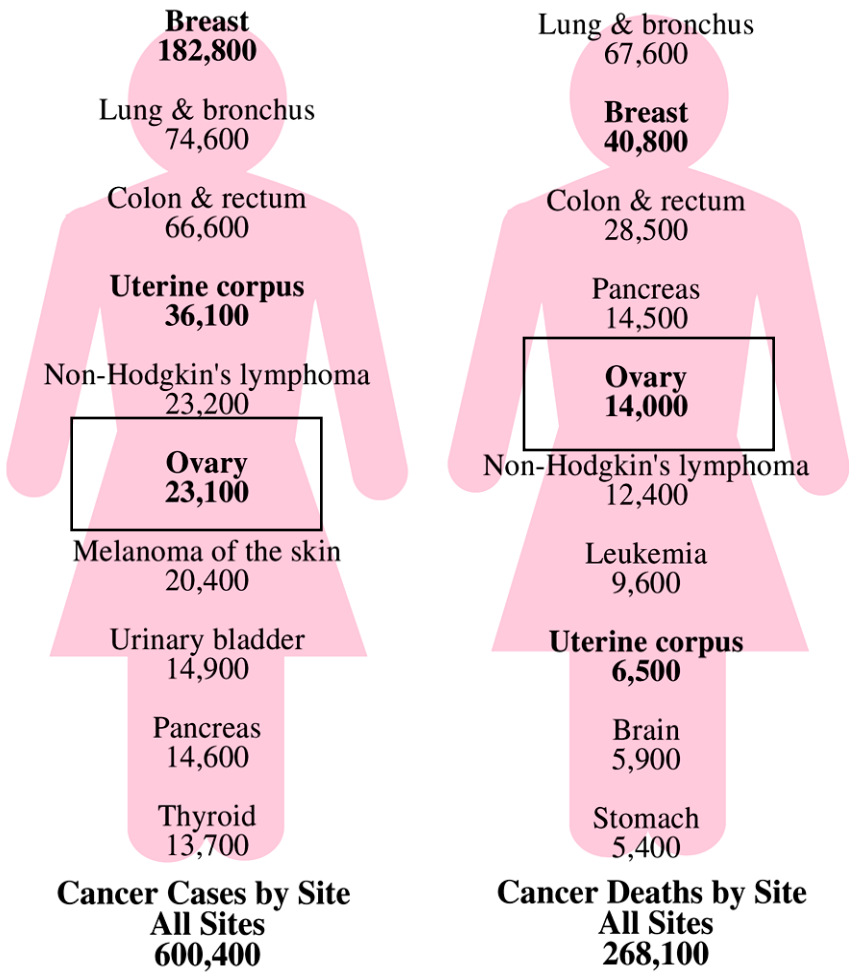
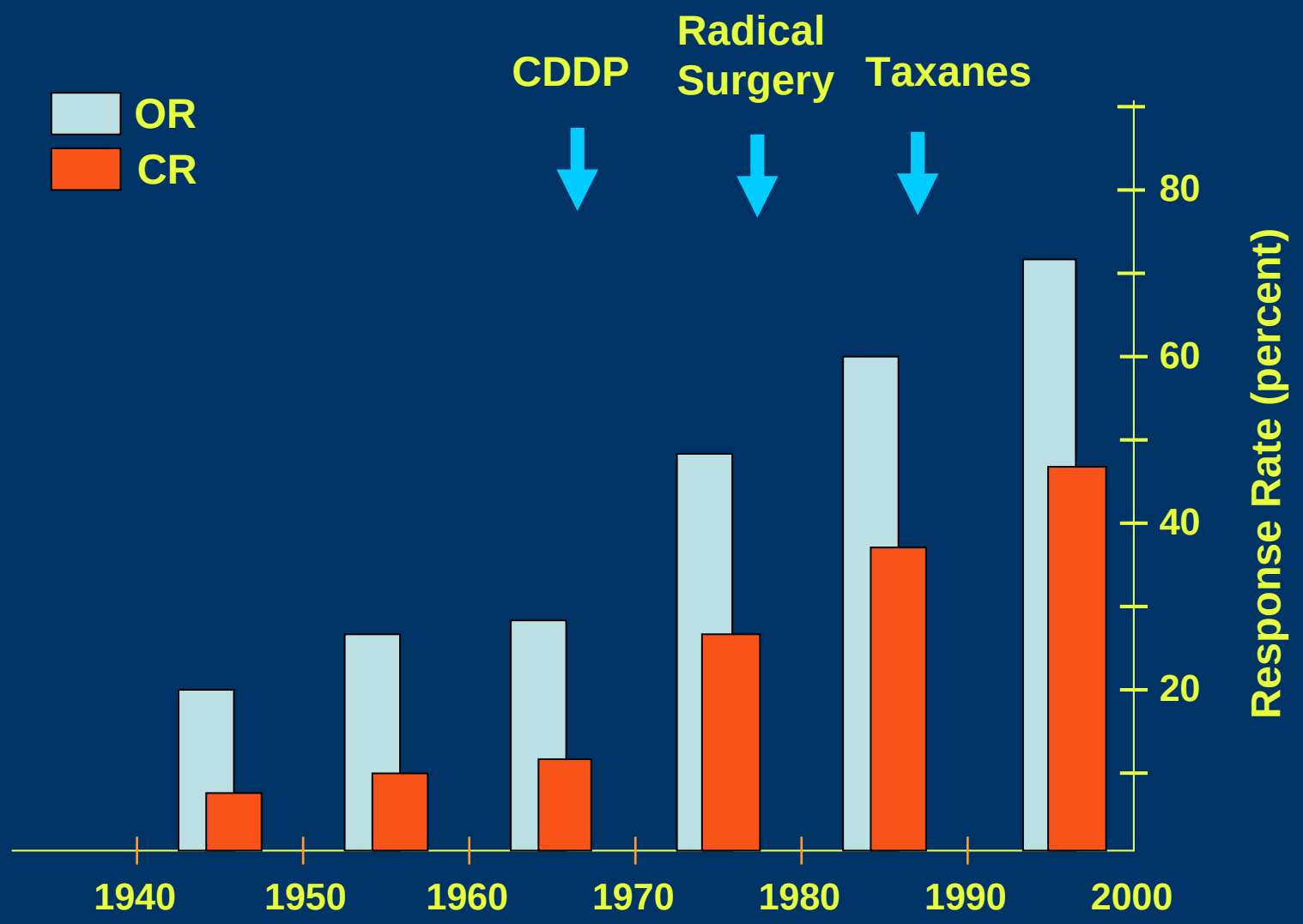
Ovarian Cancer Outcomes

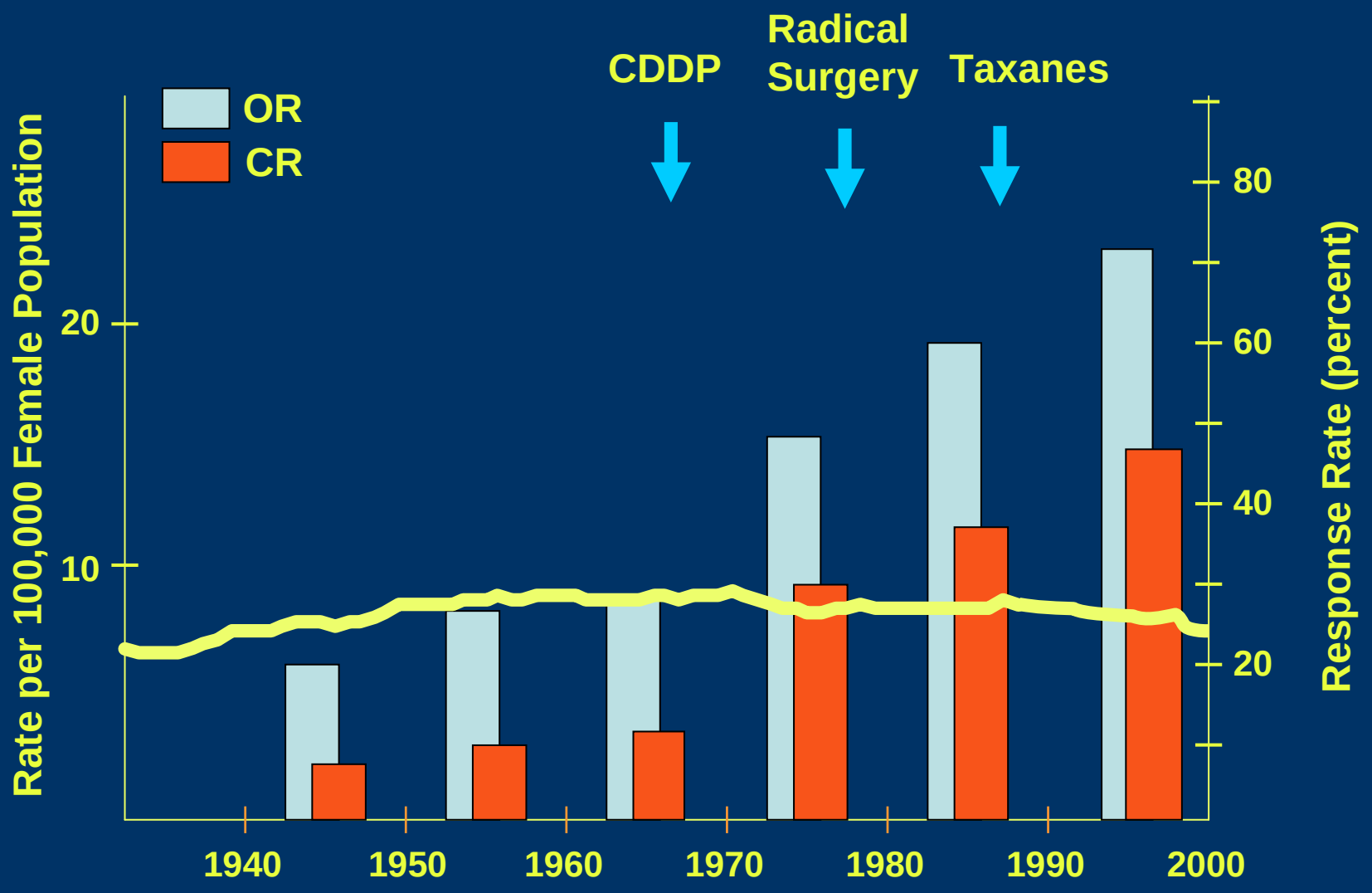
American Cancer Society
Female Cancers 2000 Statistics



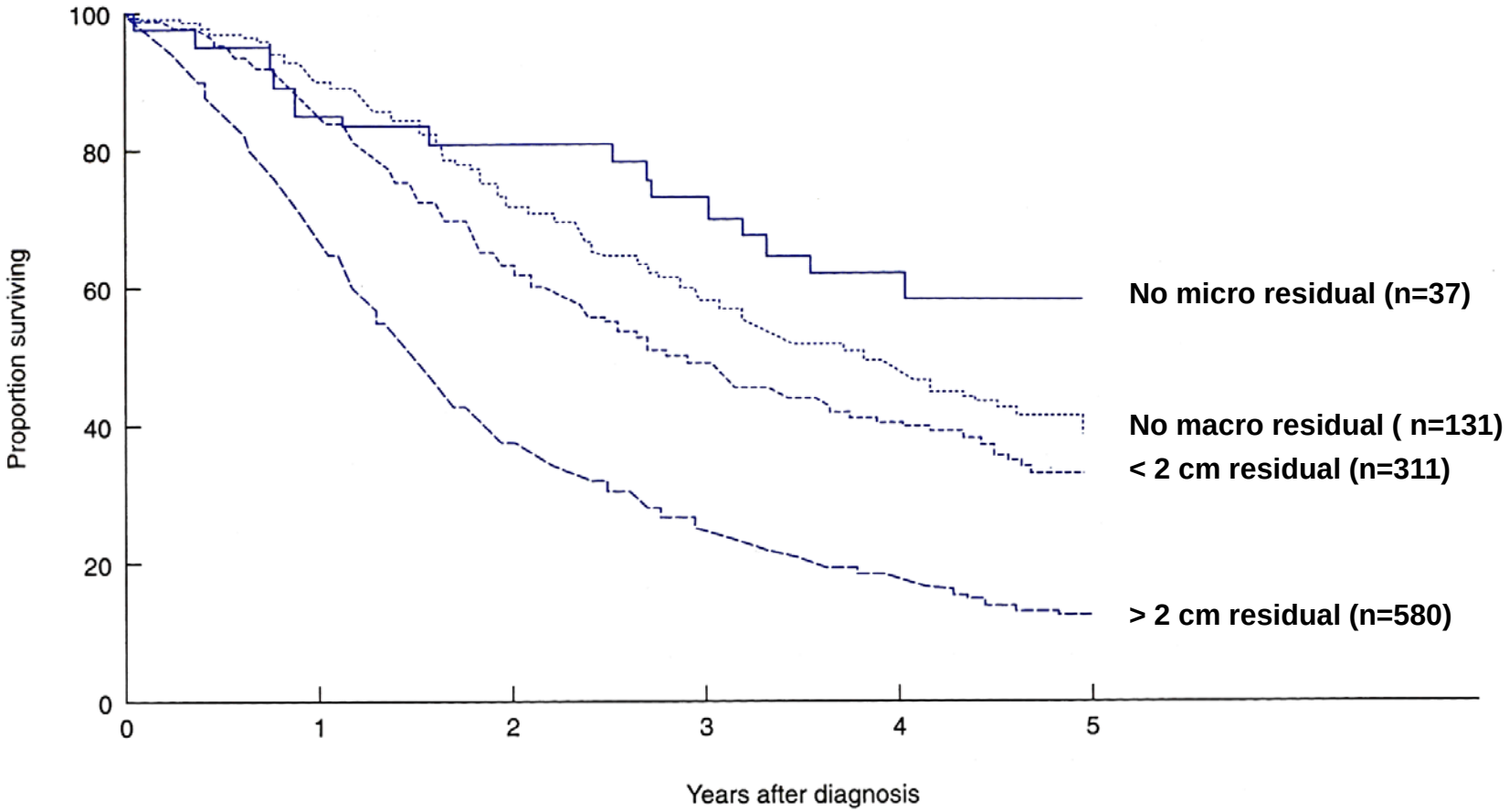
Response Rates in U.S. Women with Ovarian Cancer



Age-Adjusted Cancer Death Rates vs. Response Rates in U.S. Women with Ovarian Cancer

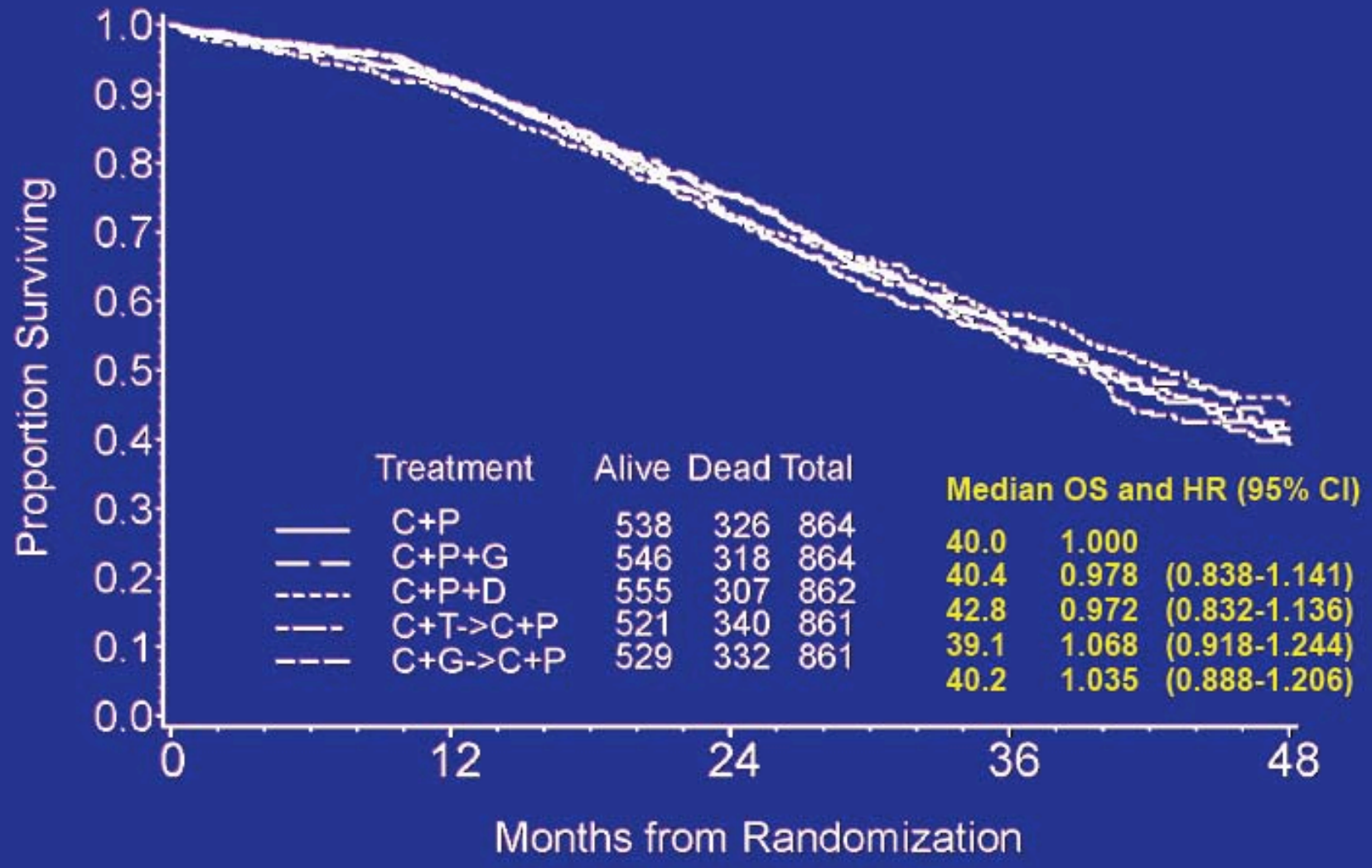


5-yr Survival in Stage IIIc Ovarian Ca (1059 Patients Treated in 1992-1994)

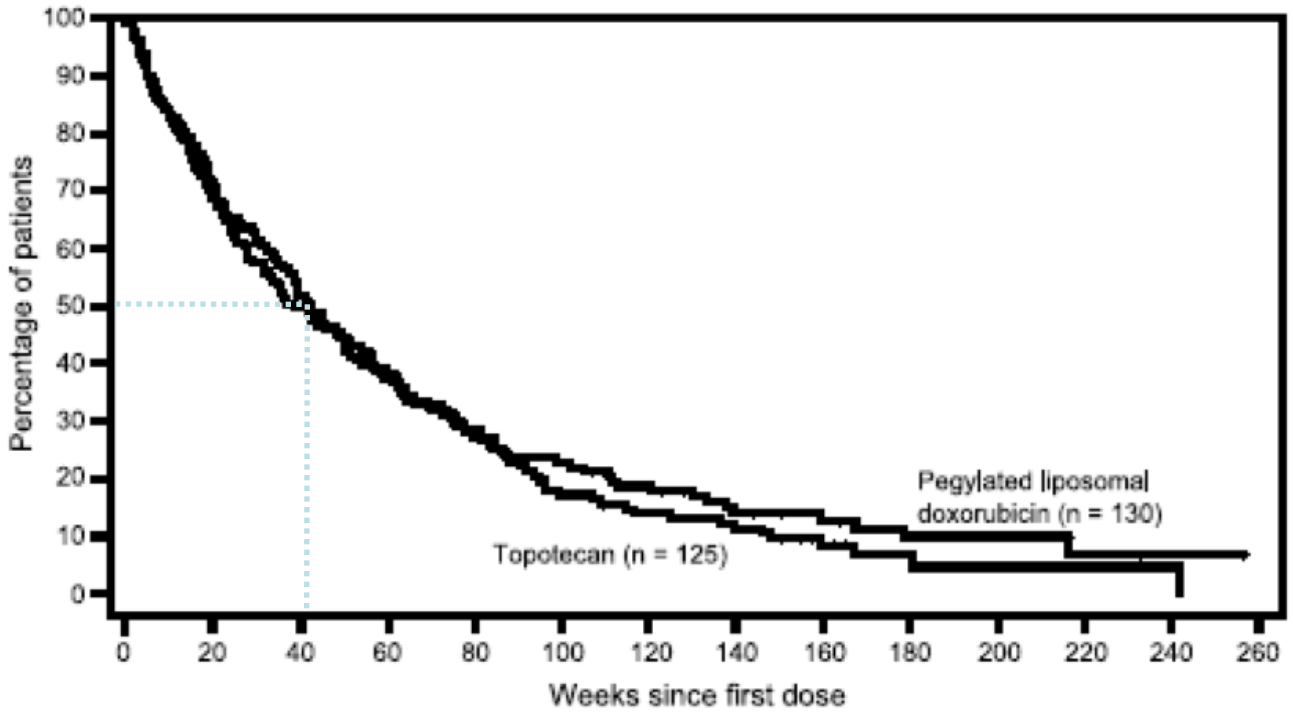


FIGO Annual Report 1998

Chemotherapy Intensification Does not Help



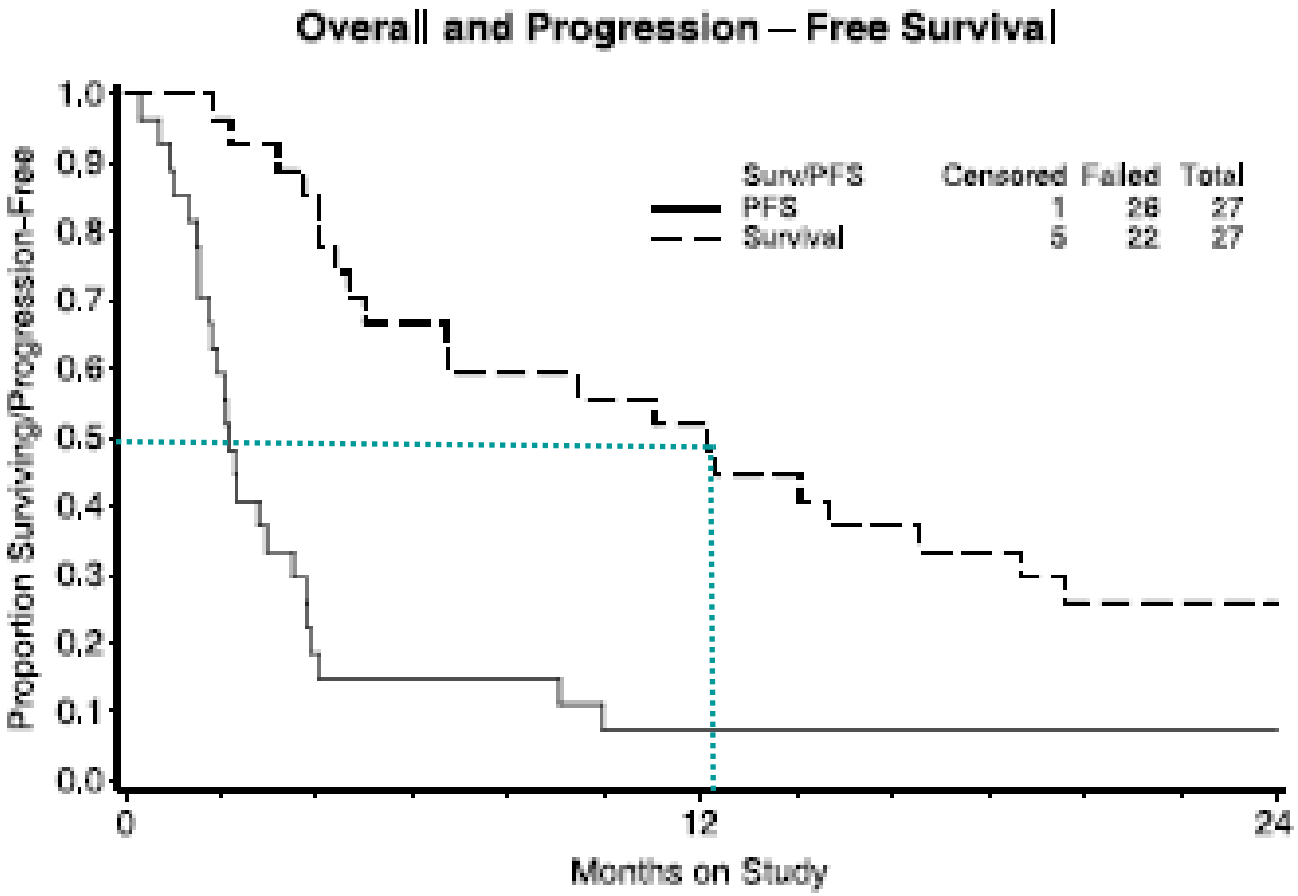
Overall Survival of Patients with Recurrent Ovarian Cancer



Gordon et al, Gyn Oncol 2004

Median Survival <10 months

Phase II Study of Gefitinib in Patients with Relapsed or Persistent Ovarian or Primary Peritoneal Carcinoma and Evaluation of Epidermal Growth Factor Receptor Mutations and Immunohistochemical Expression: A Gynecologic Oncology Group Study



Median Survival = 12 months

Rationale for Immunotherapy in Ovarian Cancer

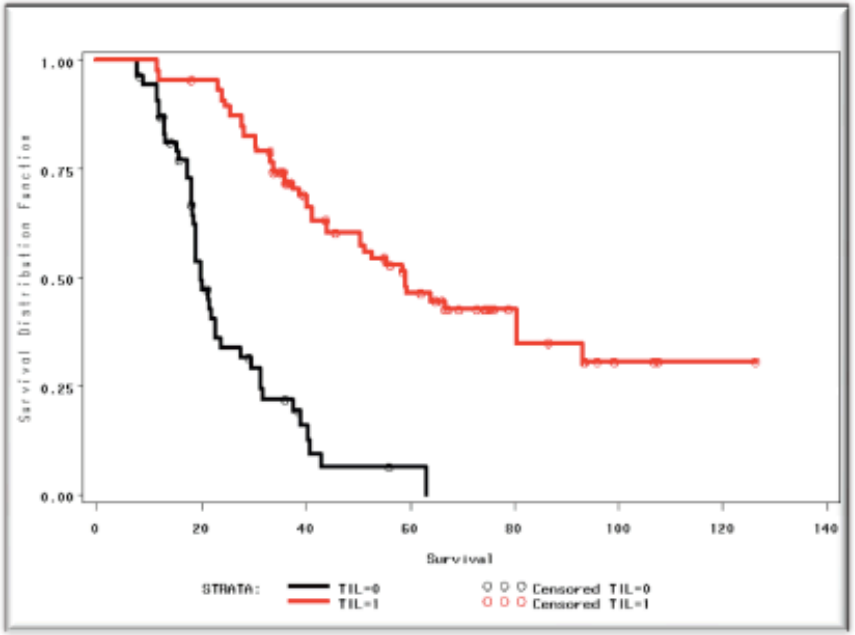
Impact of Intraepithelial T cells on Outcome in Ovarian Cancer Stage III/IV

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer

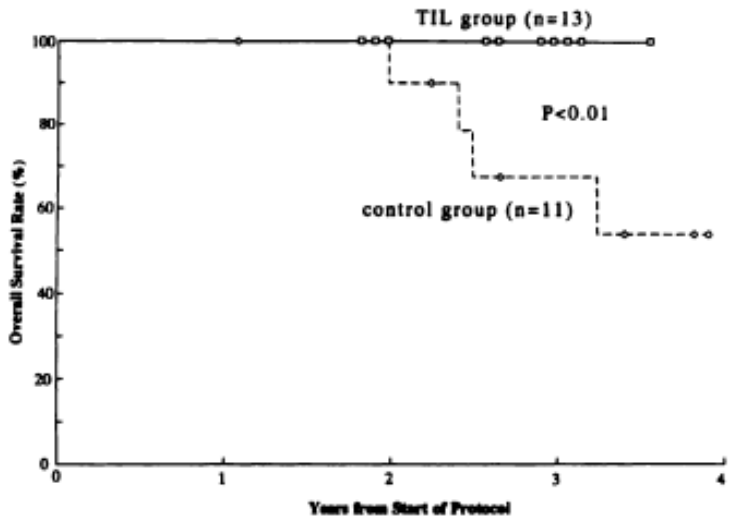
Lin Zhang, M.D., Jose R. Conejo-Garcia, M.D., Ph.D., Dionyssios Katsaros, M.D., Ph.D., Phyllis A. Gimotty, Ph.D., Marco Massobrio, M.D., Giorgia Regnani, M.D., Antonis Makrigiannakis, M.D., Ph.D., Heidi Gray, M.D., Katia Schlienger, M.D., Ph.D., Michael N. Liebman, Ph.D., Stephen C. Rubin, M.D., and George Coukos, M.D., Ph.D.

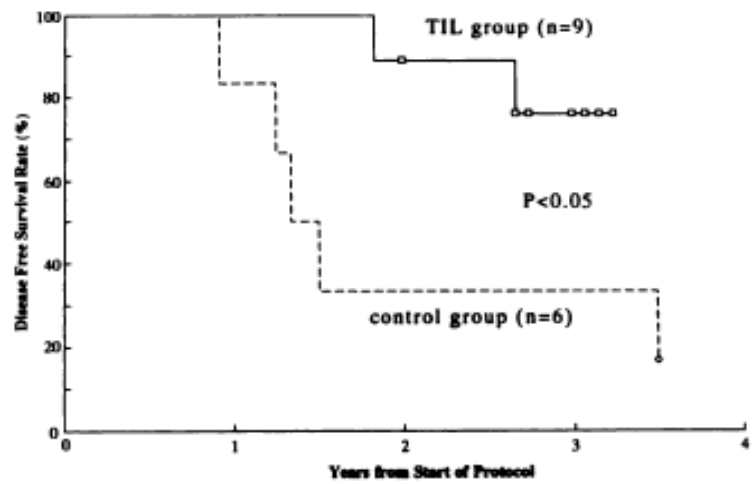


Prolonged Disease-free Period in Patients with Advanced Epithelial Ovarian Cancer after Adoptive Transfer of Tumor-infiltrating Lymphocytes[1]

Kazuyuki Fujita, Hirokazu Ikarashi, Koichi Takakuwa, Shoji Kodama, Akiteru Tokunaga, Takeshi Takahashi, and Kenichi Tanaka[2]

Department of Obstetrics and Gynecology, Niigata University School of Medicine, 1, Asahimachi-dori [K. F., H. I., Ko. T., S. K., Ke. T.]; Department of Obstetrics and Gynecology, Niigata City Hospital [A. T.]; and Department of Obstetrics and Gynecology, Niigata Cancer Center, Niigata 951 [T. T.], Japan





Rationale for Targeting the alpha Folate Receptor

alpha-Folate Receptor

An attractive tumor target in epithelial ovarian cancer

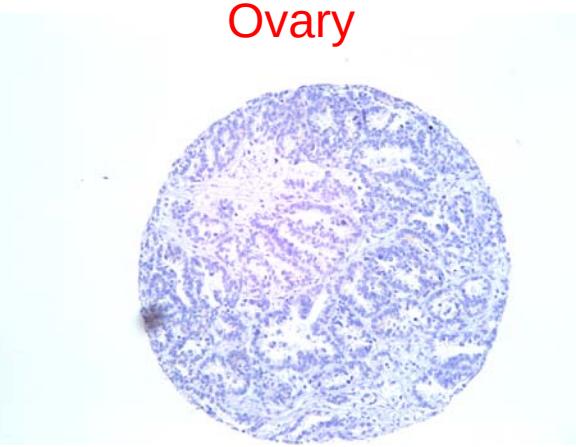
- The key to scFv-based CIR approach is the identification of antigens with high surface expression on tumor cells, but restricted expression on normal tissues.

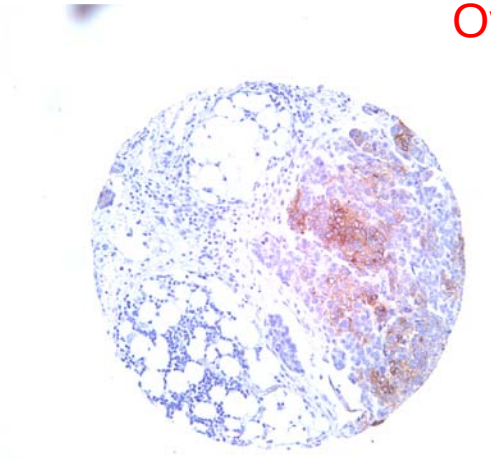
- FRa represents a strong candidate tumor antigen in the targeted therapy of epithelial ovarian cancer.

Cancers (solid tumors)	New cases per year in US	%FR	FR-expressing cancers/year
Breast	176 300	48	84 624
Lung	171 600	78	133 848
Uterus	37 400	90	33 660
Colon-rectum	129 400	32	41 408
Ovarian	25 200	90	22 680
Kidney	30 000	75	22 500
Head/neck	39 750	52	20 670
Brain and CNS	16 800	90	15 120
Gastric	26 700	38	10 146
Pancreatic	28 600	13	3718
Endocrine	19 800	14	2772
Testicular	7400	17	1258
Total	708 950		392 404

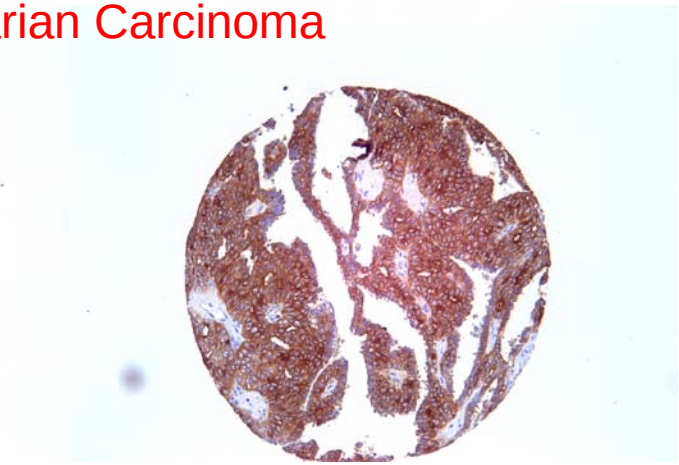
Alpha-Folate Positive Expression in Normal and Cancer Tissue

Ovary





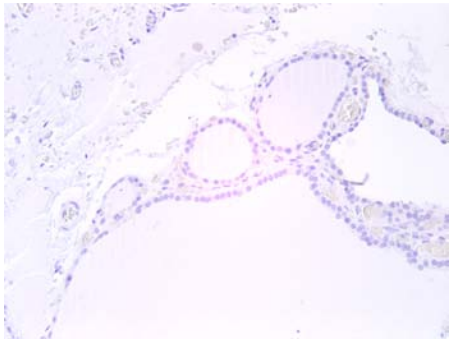
Ovarian Carcinoma



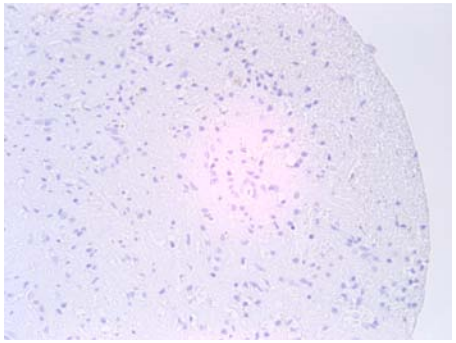
Expression of Alpha Folate Receptor is Restricted in Normal Tissues

System	Alpha Folate Receptor Expression
Central Nervous System	Choroid plexus - Luminal surface
Respiratory	Bronchi - Luminal surface
Exocrine glands	Human milk
Gastrointestinal	Submandibular salivary glands Intestinal brush-border
Genitourinary	Proximal tubules of the kidney

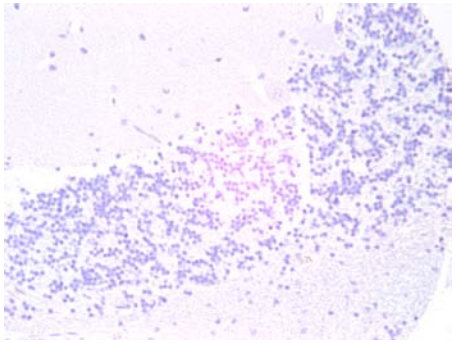
Alpha-Folate Receptor Expression in Normal Tissues



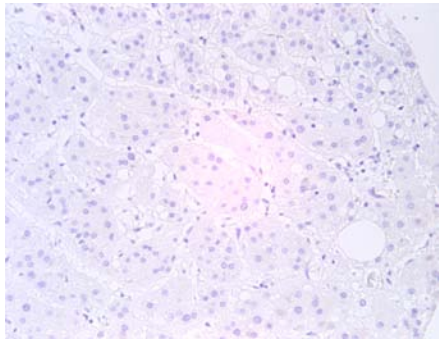
Thyroid



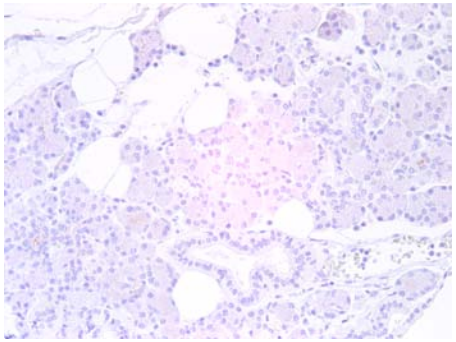
Spinal Cord



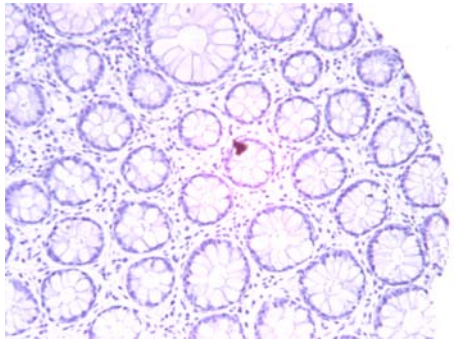
Cerebellum



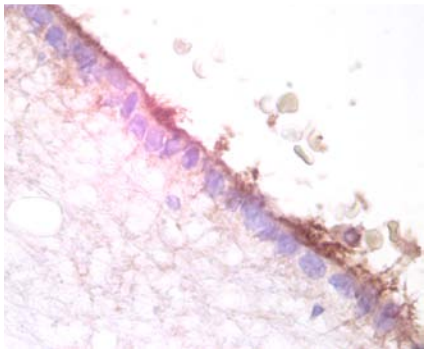
Liver



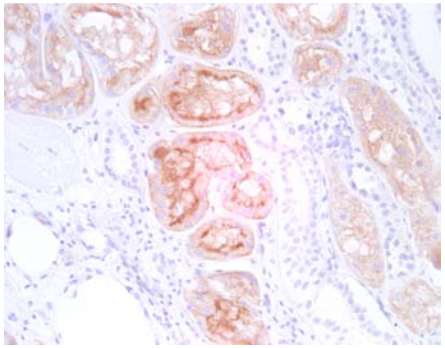
Salivary Gland



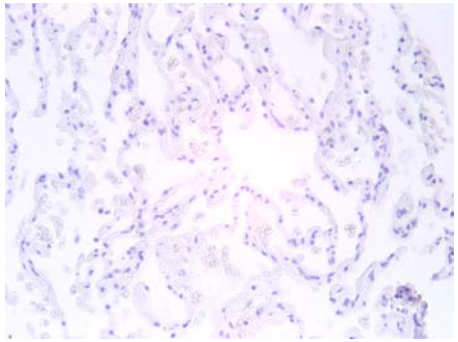
Colon



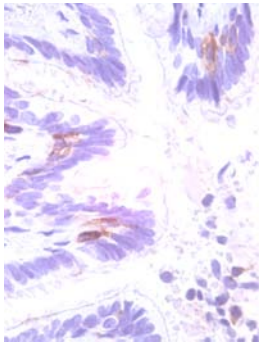
Choroid Plexus



Proximal Tubules



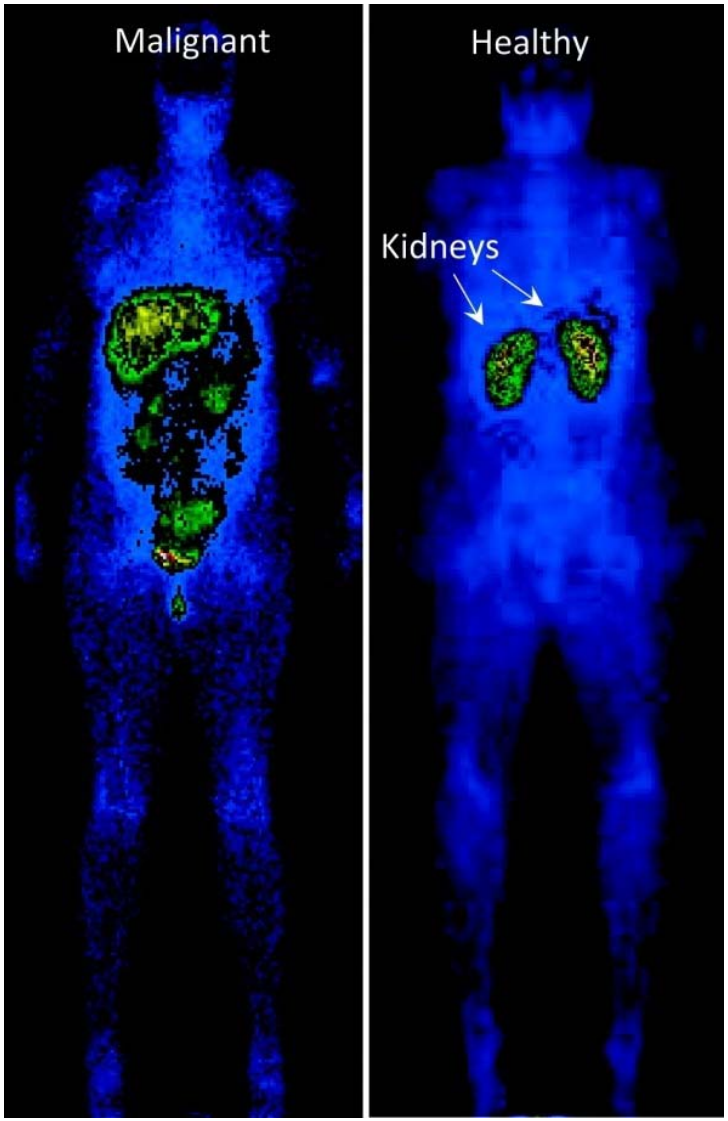
Lung Alveoli



Bronchi

Comparison of [111In]DTPA–folate uptake in a patient with stage III ovarian cancer (left) and a healthy volunteer (right).

FR expression in the kidney is limited to the apical surface of the proximal tubule where the receptor captures the vitamin (or a folate–drug conjugate) from the nascent urine and transcytoses it back across the kidney epithelium for release into the blood.(34) In this process, FR serves as a salvage receptor to prevent loss of folates in the urine. Because most folate conjugates are not retained in the kidneys, **no renal toxicity has ever been observed in animals or humans treated with folate-chemotherapeutic agent conjugates.(**35, 36)



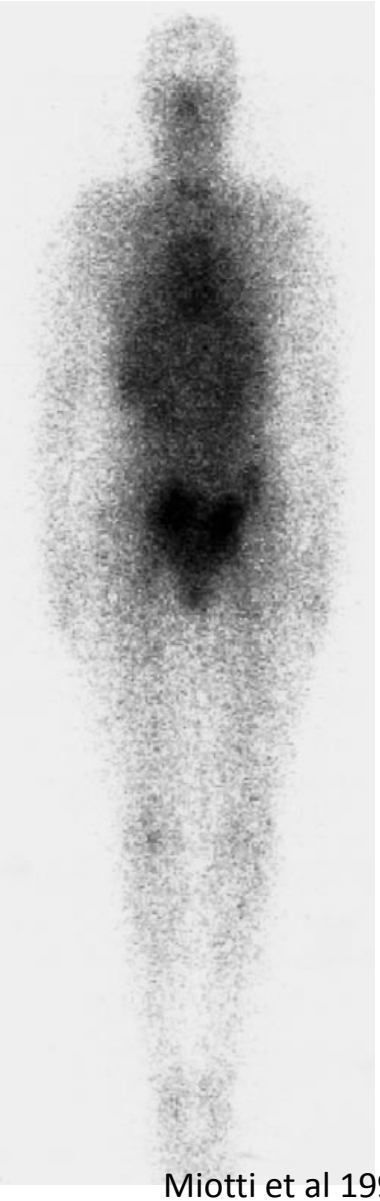
Wei Xia; Philip S. Low; *J. Med. Chem.* 2010, 53, 6811-6824.

Antibody-based detection of alpha-folate receptor

All ovarian carcinoma lesions that were found at surgery were visualized in the three patients having epithelial ovarian cancer.

After the radio-immunoconjugate had reached its maximum blood level, **no retention of activity was seen in normal organs such as liver, kidneys or bone marrow.**

I^{131} cMOv18	**i.p.**	**15-80 mg**
	i.v.	**30-75 mg**



Miotti et al 1999

Safety Data

Crippa et al, 1995

IP Radio-immune therapy with radio-labeled MOV18

16 patients with minimal residual disease ovarian cancer

5 patients achieved CR, 6 showed SD and 5 PD

Toxicity was negligible

Spannuth et al, 2010, Konner et al, 2010:

A phase I dose-escalation study using intravenous Farletuzumab (a humanized monoclonal antibody against FRα)

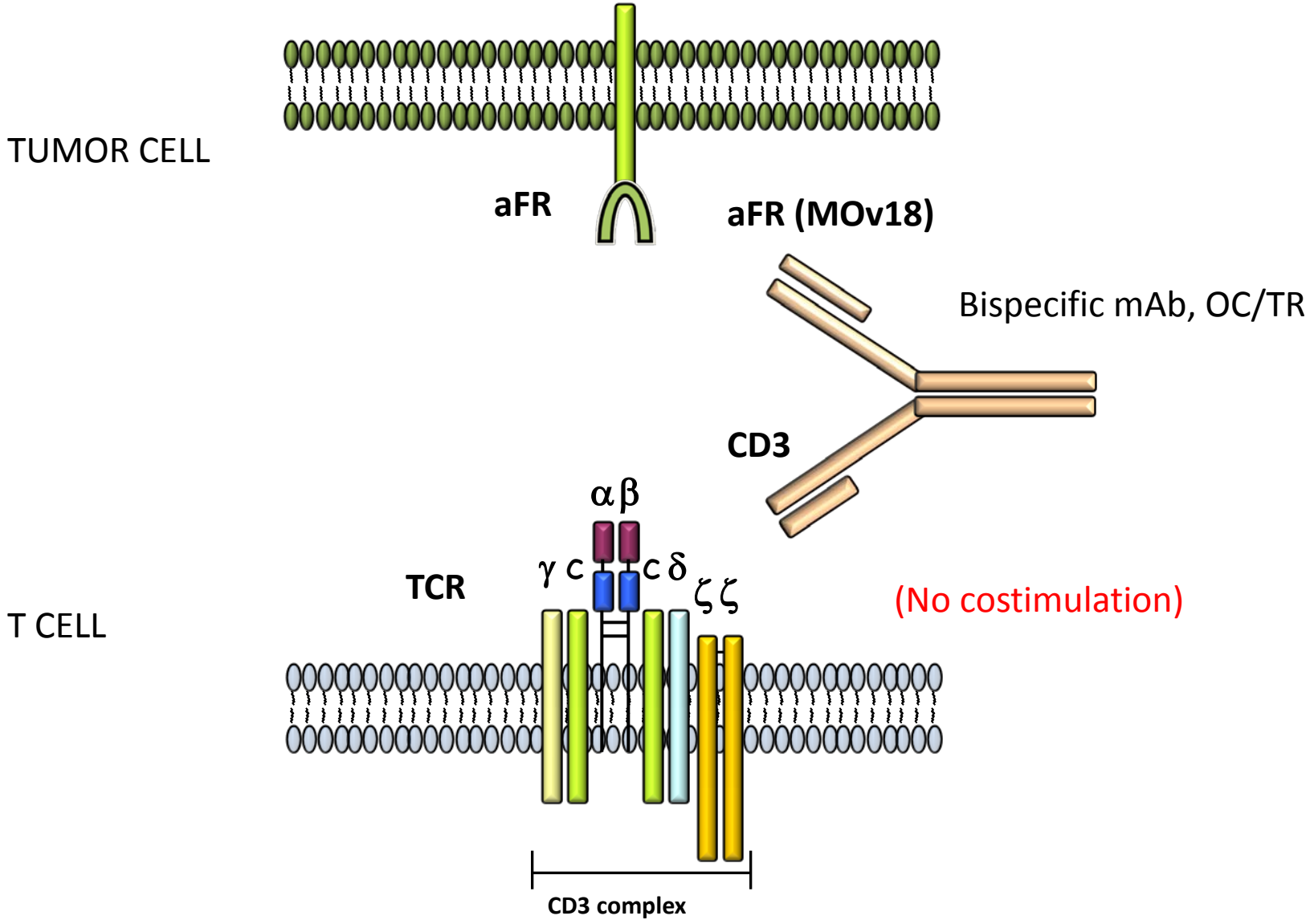
25 patients were included in the safety, efficacy, and PK analyses.
9 patients with SD and 4patients with decreased CA125 levels

No DLTs or MTDs. No serious or severe (≥Grade 3) (Treatment Related Adverse Events) TRAEs and no treatment-related myelotoxicity or neurotoxicity.

The most common TRAEs were hypersensitivity reactions (15 patients; 60%), fatigue (12 patients; 48%), and diarrhea (4 patients; 16%).

Adoptive Therapy Targeting the αFR

Retargeting of T lymphocytes using F(ab')2 fragments of the bispecific monoclonal antibody (MAb) OC/TR, which is directed to the CD3 molecule on T lymphocytes and to the folate receptor on ovarian carcinoma cells. (Canevari et al)



Regression of advanced ovarian carcinoma by intraperitoneal treatment with autologous T lymphocytes retargeted by a bispecific monoclonal antibody.

28 patients treated with MRD after surgical debulking

2 cycles of 5 daily i.p. infusions + IL-2; 11pts received second daily infusion + IL-2

27% response rate; 3CR, 3PR, 1MR, 7SD, 12 PD

Immunotherapy-related toxic effects included mild to moderate
-fever, nausea, emesis, and fatigue.

<u>Limitations:</u>

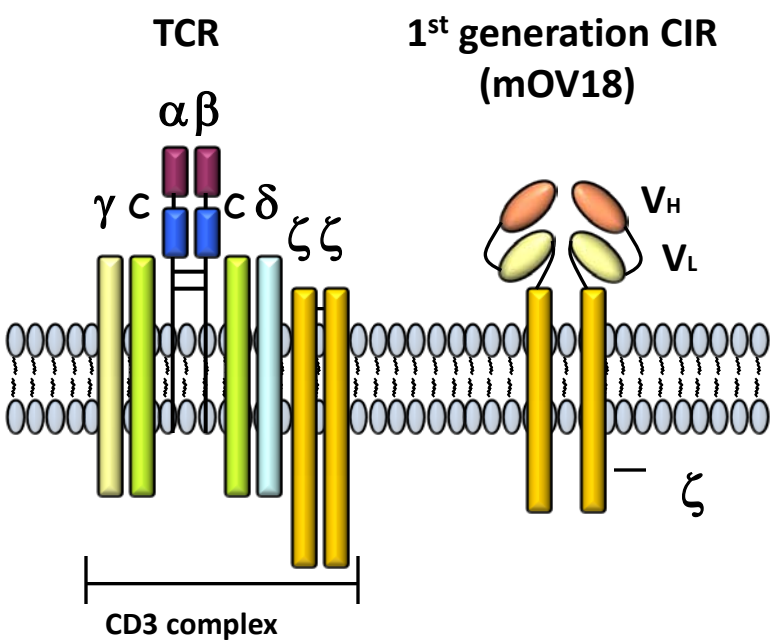
88% (21/25) of patients receiving bispecific AB-loaded T cells developed HAMA

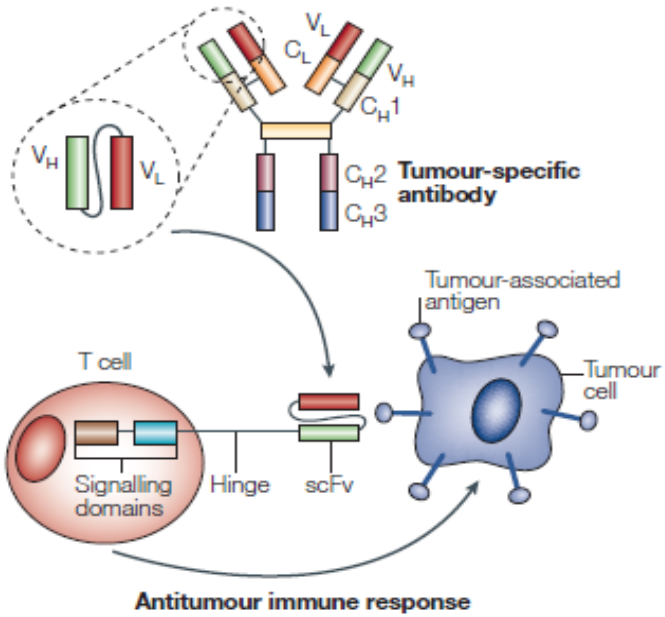
T cells require pre-activation

Stability of bound bi-mAb; inability to generate memory

Canevari et al. JNCI,1995

Chimeric Immune Receptor (CIR)





Michael H. Kershaw,et al. Nat Rev Immunol.2005

Provides stable expression of the MOv18 scFv
HLA-independent
Recognizes surface protein; antigen processing not required

A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer

Pts received autologous or allogeneic T cells expressing aFR CIR + IL-2 without lymphodepleting preconditioning

No responses observed

T cells did not persist long term and generate memory

T cells did not track to tumor

Toxicity related to IL-2 administration

An inhibitory factor developed in the serum

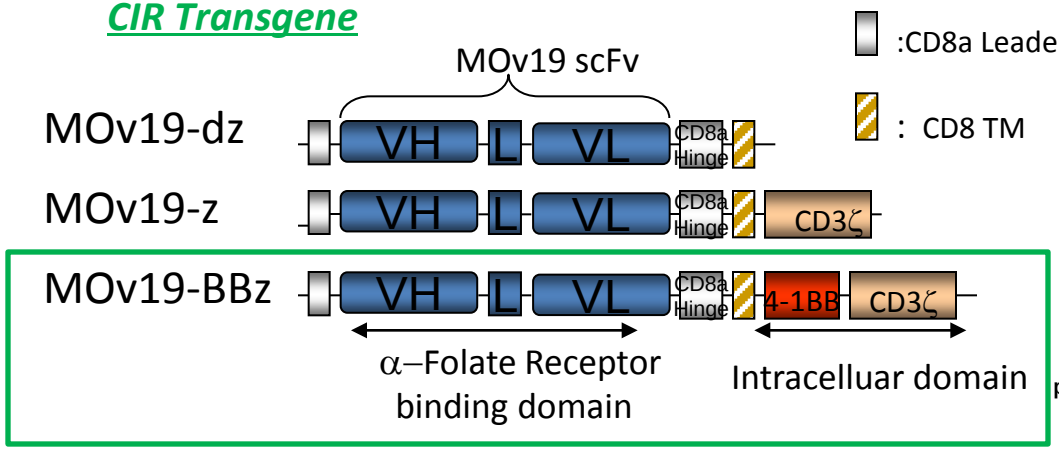
"Future studies need to employ strategies to extend T cell persistence."

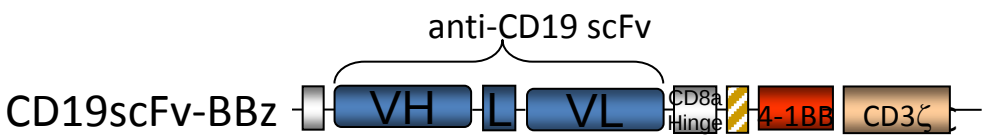
Kershaw et al. Clin Can Res, 2006

Preclinical data with pELNS-MOv19-4-1BB-CD3zeta

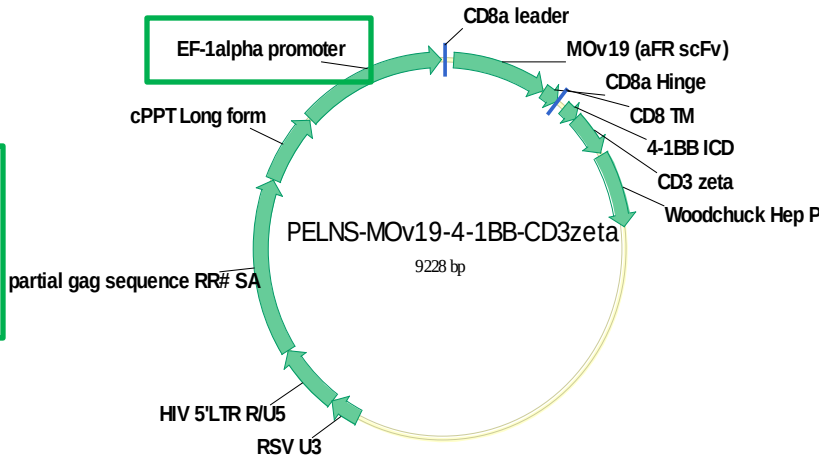
Construction of alpha-folate receptor-specific chimeric immune receptor

CIR Transgene





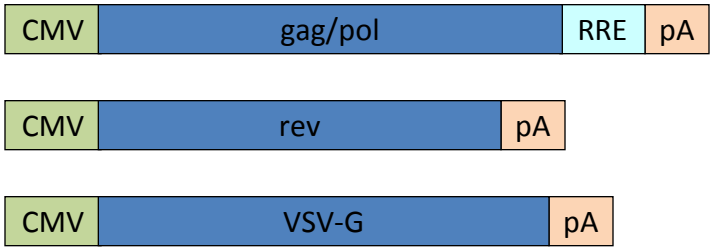
Vector



Plasmid map of PELNS—MOv19-4-1BB-CD3z

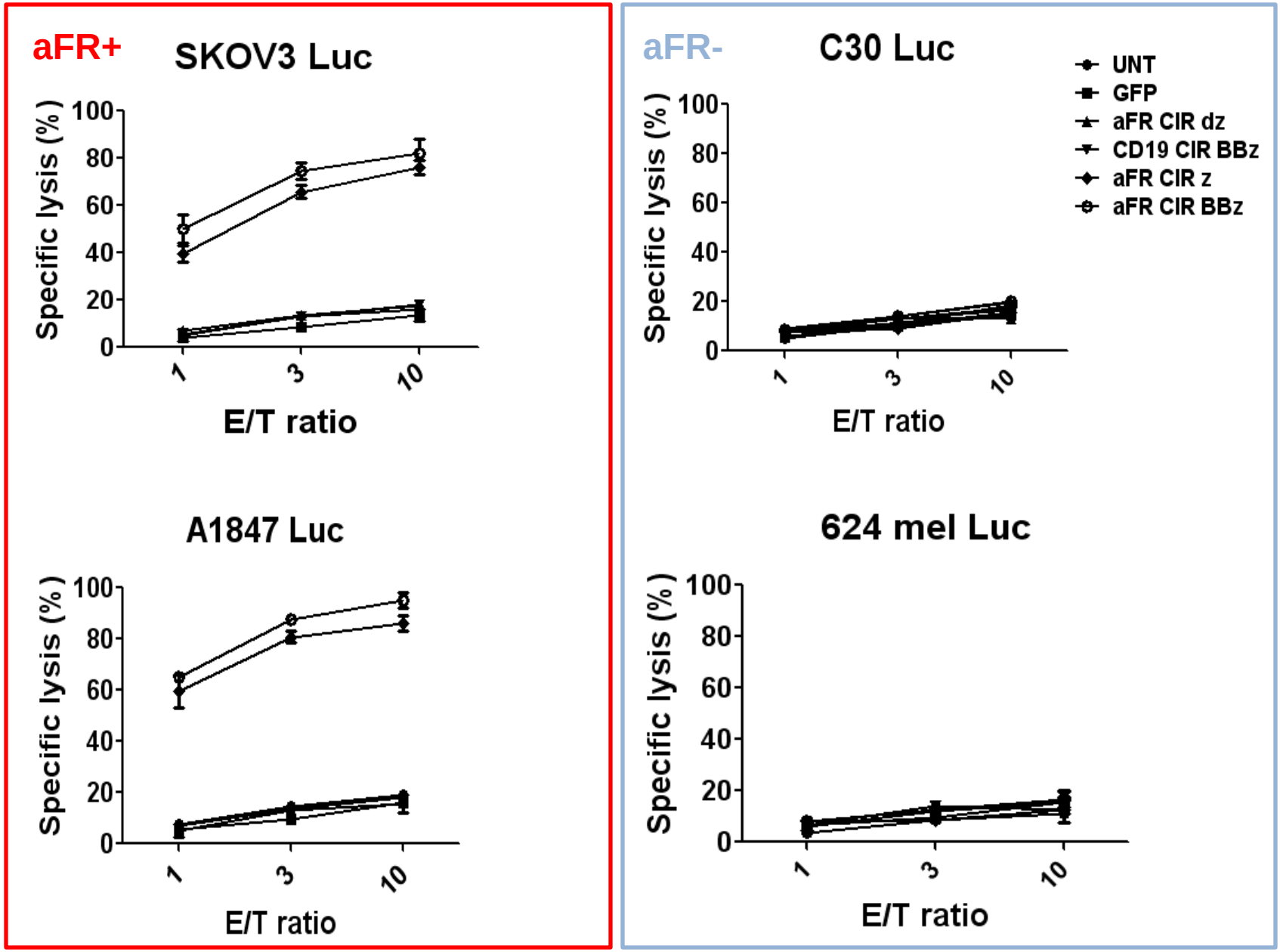
GMP plasmid is manufactured at Nature Technology

Packaging Plasmids



GMP vectors are manufactured at City of Hope

aFR CIR-BBz TRANSDUCED T CELLS KILL aFR+ OVARIAN CANCER CEL

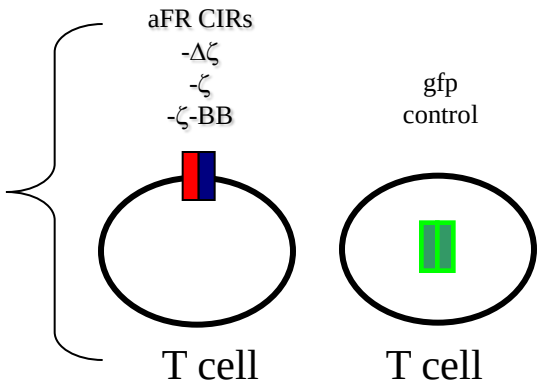


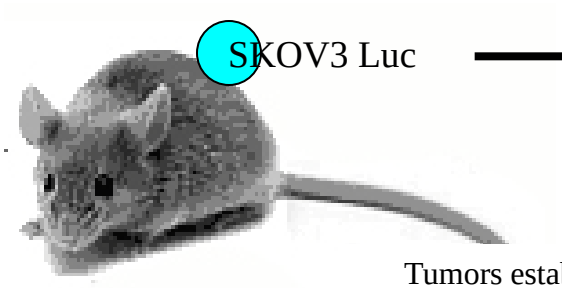
PRECLINICAL MODEL: treatment of large, established human ovarian cancer using CIR gene therapy

aFR is an important tumor target; over-expressed by 90% of ovarian cancers.

Human tumor inoculation:
3e6 αFR+ luciferase+ Ovarian Cancer (SKOV3) cells s.c.

I.T. administration of αFR-specific CIR Engineered T cells



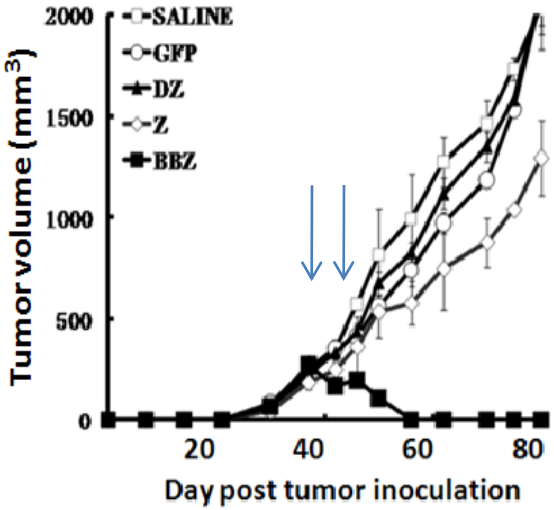


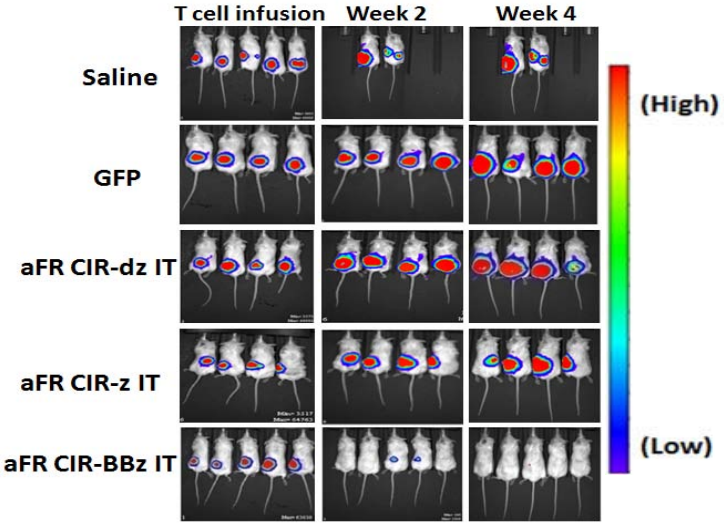
NOD/SCID/γ-chain-/- mouse

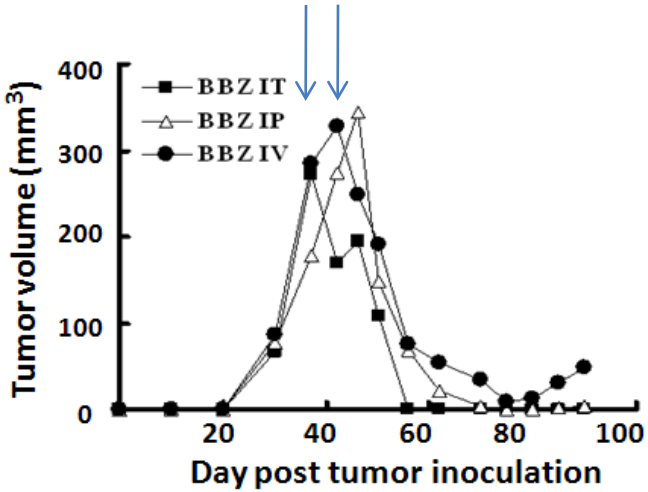
Tumors established for ~30 days
(~250mm^3 tumor volume)

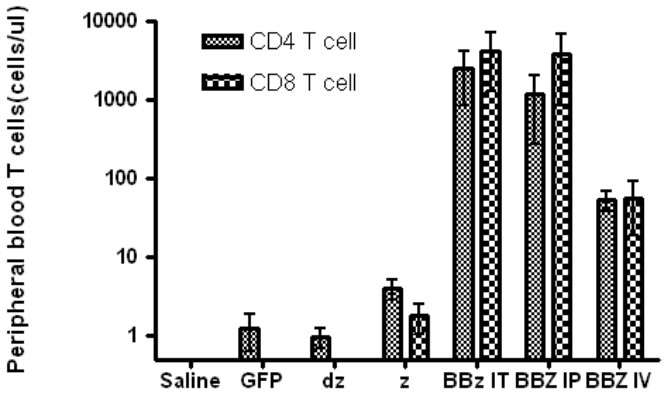
Measurement of tumor size with caliper/imaging

ANTITUMOR EFFICACY AND IMPROVED PERSISTENCE OF aFR-BBz CIR TRANSDUCED T CELLS





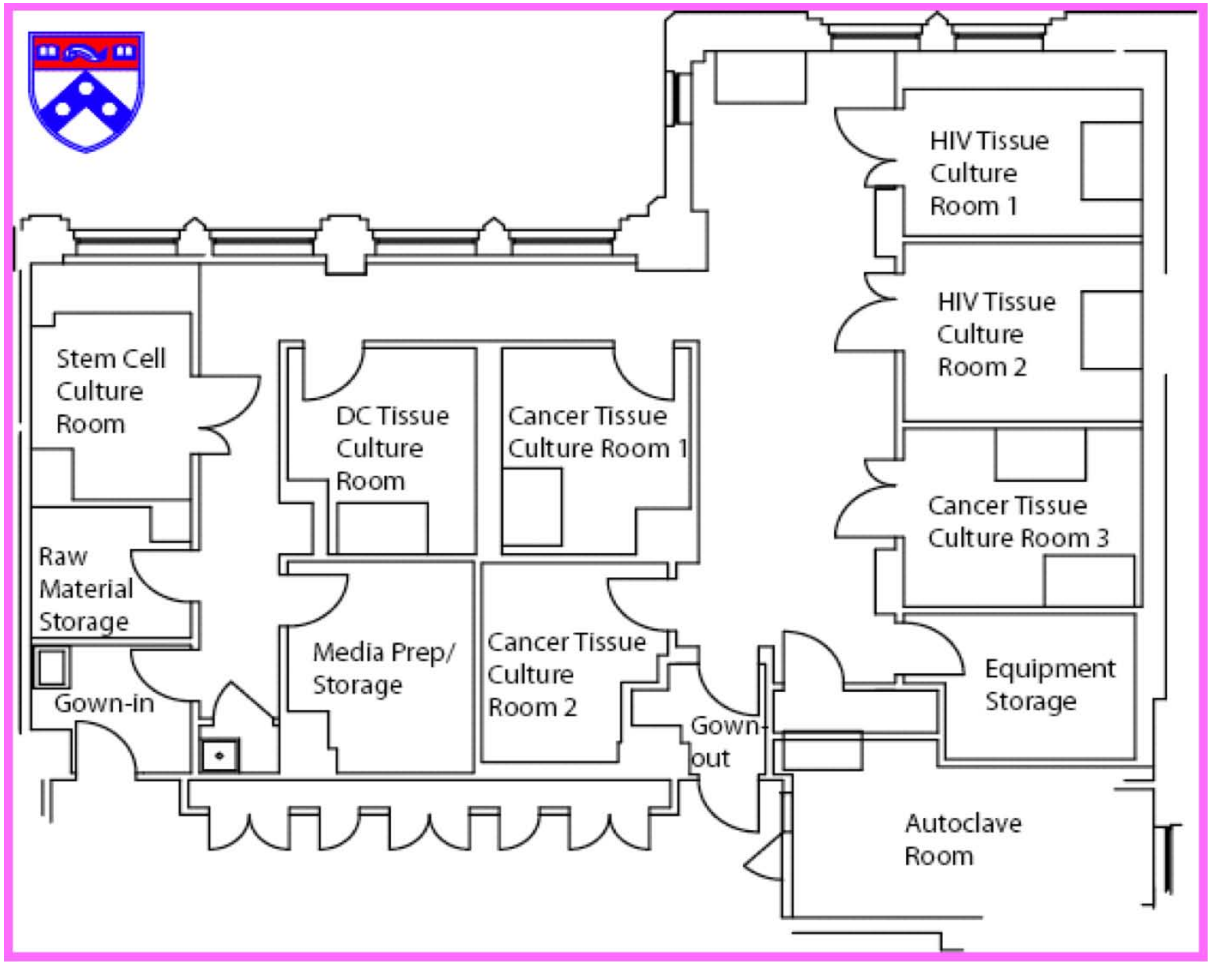


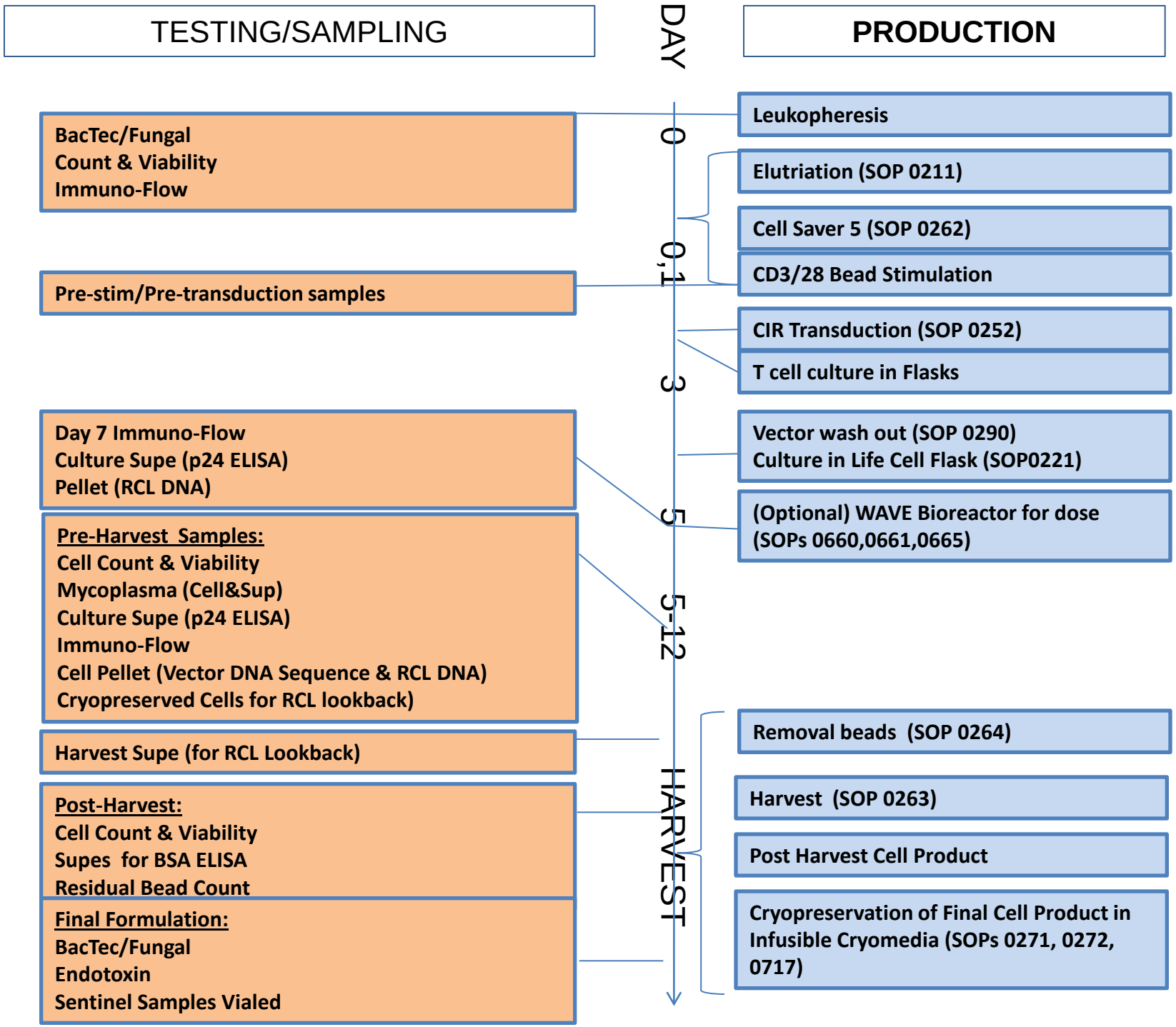


Clinical trial design

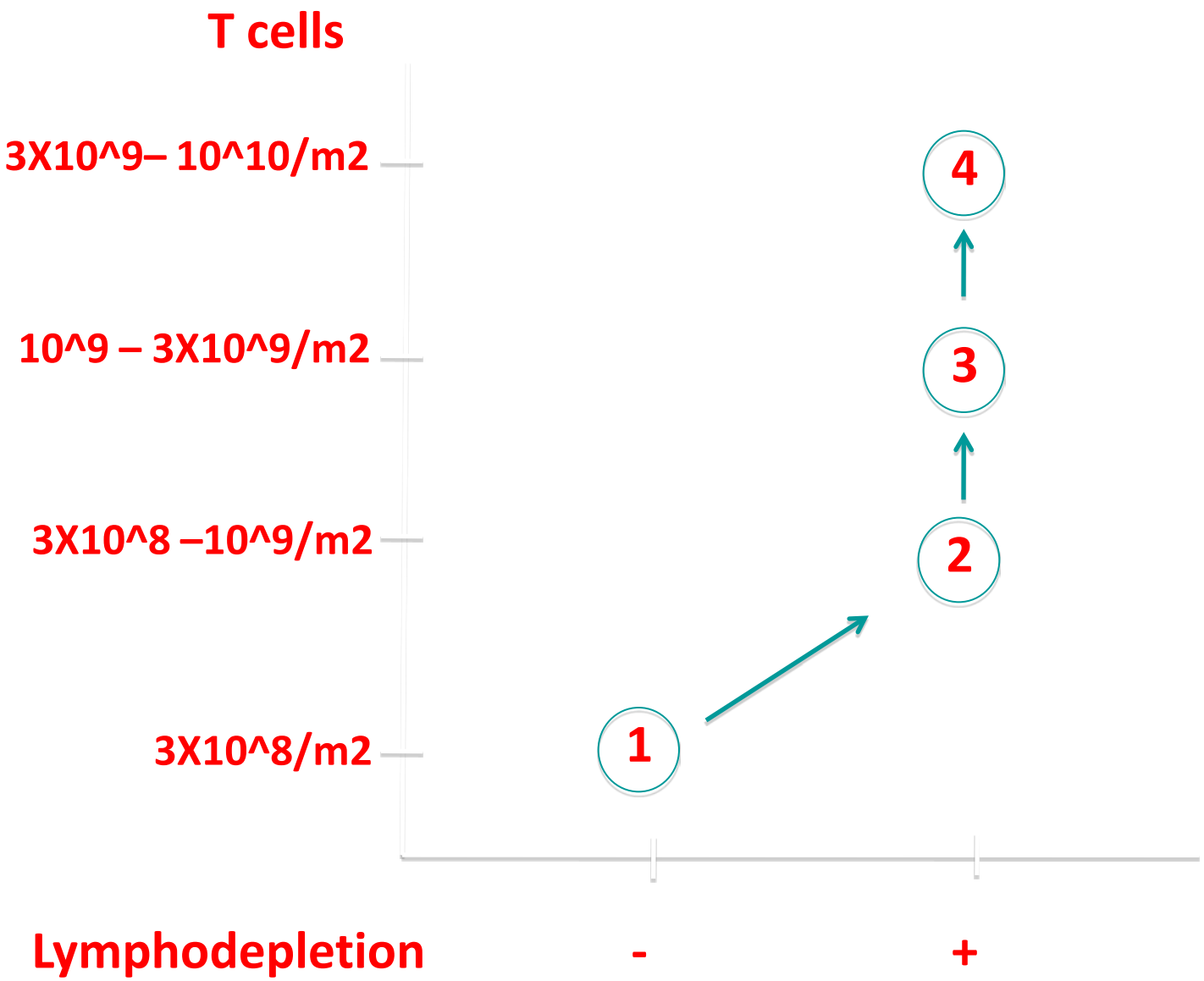
Clinical Production of aFR CIR T Lymphocytes for Therapy in PENN's CVPF

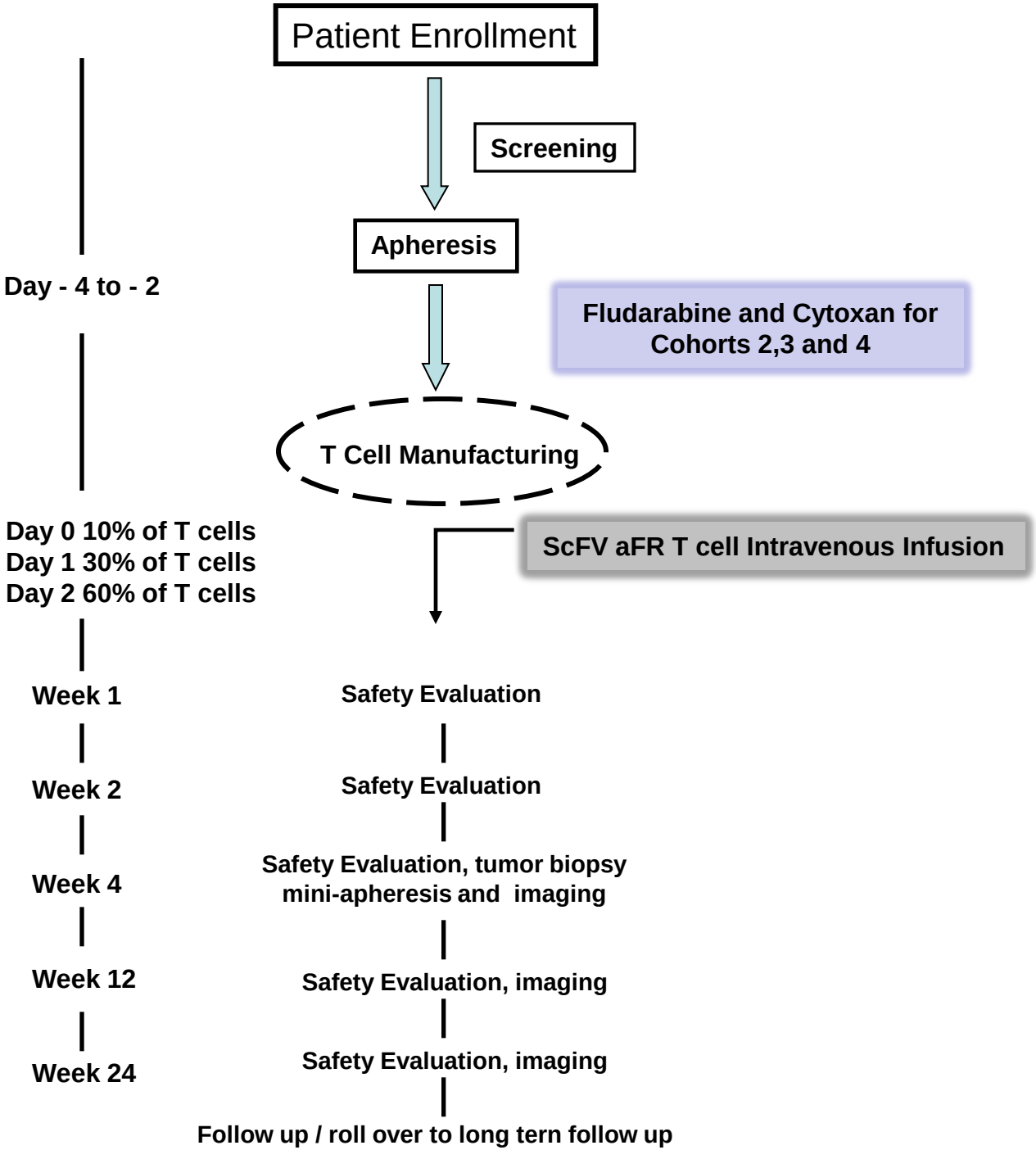
- **FACT Accredited**
- **NCI CCSG "Exceptional Scoring"**




TESTING/SAMPLING
DAY
PRODUCTION
BacTec/Fungal
Count & Viability
Immuno-Flow
0
Leukopheresis
Elutriation (SOP 0211)
Cell Saver 5 (SOP 0262)
0,1
CD3/28 Bead Stimulation
Pre-stim/Pre-transduction samples
CIR Transduction (SOP 0252)
T cell culture in Flasks
3
Day 7 Immuno-Flow
Culture Supe (p24 ELISA)
Pellet (RCL DNA)
Vector wash out (SOP 0290)
Culture in Life Cell Flask (SOP0221)
5
(Optional) WAVE Bioreactor for dose
(SOPs 0660,0661,0665)
Pre-Harvest Samples:
Cell Count & Viability
Mycoplasma (Cell&Sup)
Culture Supe (p24 ELISA)
Immuno-Flow
Cell Pellet (Vector DNA Sequence & RCL DNA)
Cryopreserved Cells for RCL lookback)
5-12
Harvest Supe (for RCL Lookback)
Removal beads (SOP 0264)
Post-Harvest:
Cell Count & Viability
Supes for BSA ELISA
Residual Bead Count
HARVEST
Harvest (SOP 0263)
Post Harvest Cell Product
Final Formulation:
BacTec/Fungal
Endotoxin
Sentinel Samples Vialed
Cryopreservation of Final Cell Product in Infusible Cryomedia (SOPs 0271, 0272, 0717)

Trial Design




Patient Enrollment
Screening
Apheresis
Day - 4 to - 2
Fludarabine and Cytoxan for Cohorts 2,3 and 4
T Cell Manufacturing
Day 0 10% of T cells
Day 1 30% of T cells
Day 2 60% of T cells
ScFV aFR T cell Intravenous Infusion
Week 1
Safety Evaluation
Week 2
Safety Evaluation
Week 4
Safety Evaluation, tumor biopsy mini-apheresis and imaging
Week 12
Safety Evaluation, imaging
Week 24
Safety Evaluation, imaging
Follow up / roll over to long tern follow up

Study Objectives

Primary Objective

Determine the safety and feasibility of the chimeric immune receptor (CIR) T cells transduced with the anti-α-FR single chain Fv fragment (scFv) administered IV alone or following transient lymphodepletion with cyclophosphamide / fludarabine

Secondary Objectives

• Define an optimal biologic dose (OBD) of anti-α-FR scFv autologous T cells and evaluate the safety profile and engraftment of CIR T cells in circulation and tumor biopsies.

• Assess the distribution of progression-free survival, overall survival and time to progression

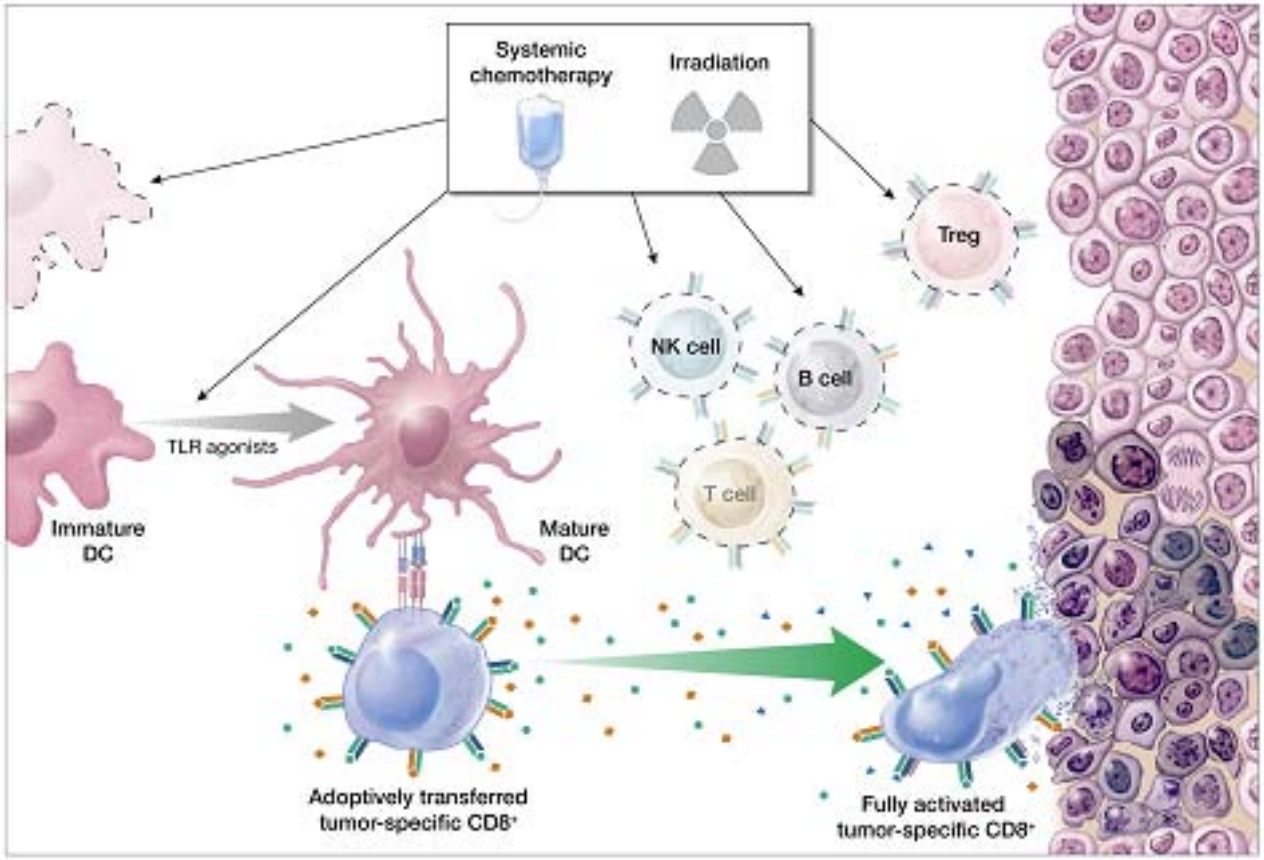
•Determine the effect of CIR T cells on tumor immunity and -α-FR expression.

• Assess the development of host immune responses to the CIR T cells and correlate with loss of engraftment or CIR T cells.

• Assess immune related-responses and correlate with expression of α-FR

Answers to RAC reviewers' questions

Lymphodepletion provides activating cues



IL-2Rα
IL-7Rα
IL-15Rα
common β chain
common γ chain

IL-2
IL-7
IL-15
IL-10

TGFβ
granzymes
CD28
CD80\86

tumor antigen
competing antigen
tumor specific TCR
competing TCR

Gattinoni, Powell, Restifo, Rosenberg, Nature Reviews Immunology 2006

Regulatory T cells in Adoptive Immunotherapy

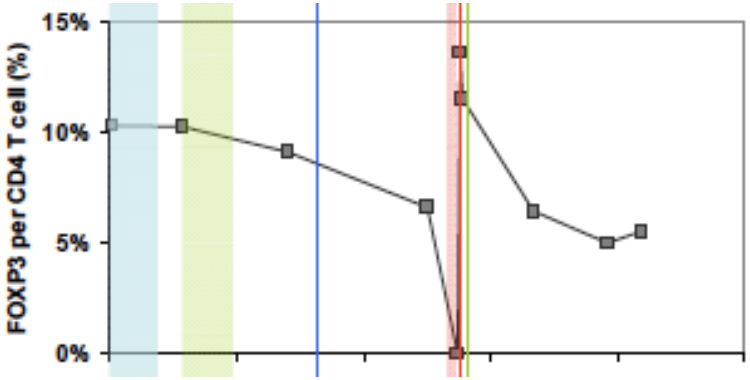
CD8+ T cell immunity against a tumor/self-antigen is augmented by CD4+ T helper cells and hindered by naturally occurring T regulatory cells. Antony PA, et al. J. Immunol. 2005 Mar 1;174(5):2591-601.

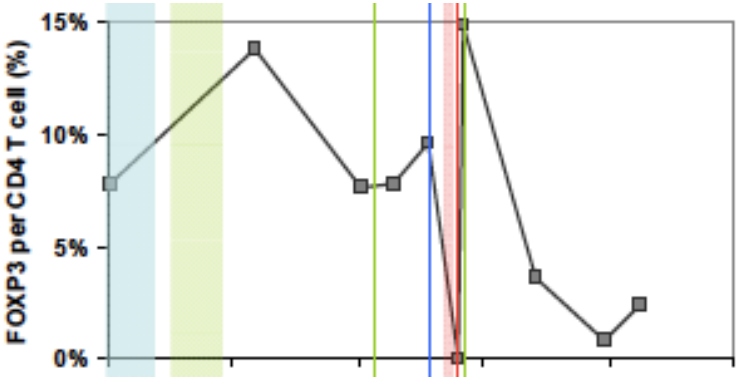
Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. Kochenderfer JN, et al. Blood. 2010 Nov 18;116(20):4099-102. Epub 2010 Jul 28.

Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Johnson LA, et al. Blood. 2009 Jul 16;114(3):535-46. Epub 2009 May 18.

Large-scale depletion of CD25+ regulatory T cells from patient leukapheresis samples. Powell DJ Jr, Parker LL, Rosenberg SA. J Immunother. 2005 Jul-Aug;28(4):403-11.

Frequency of FoxP3+ Treg after Adoptive Transfer of CD3/CD28-costimulated PBL following Lymphpodepletion





Transgene Immunogenicity in Adoptive Immunotherapy

A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Kershaw MH, et al. Clin Cancer Res. 2006 Oct 15;12(20 Pt 1):6106-15.

Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. Kochenderfer JN, et al. Blood. 2010 Nov 18;116(20):4099-102. Epub 2010 Jul 28.

Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Johnson LA, et al. Blood. 2009 Jul 16;114(3):535-46. Epub 2009 May 18.

Rapid isolation of high-affinity human antibodies against the tumor vascular marker Endosialin/TEM1, using a paired yeast-display/secretory scFv library platform.
Zhao A, et al. J Immunol Methods. 2010 Sep 15.