Targeting Tumor Antigens by Redirecting T cells using Bispecific Antibodies

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Clinical Problem

- Patients with metastatic breast cancer, hormone refractory prostate cancer, and other cancers have limited clinical options.
- Chemotherapy, irradiation, or high dose chemotherapy have become dose-limiting.
- New non-toxic strategies are needed to provide an anti-tumor effect without enhancing treatment toxicities.

Perspective

Earlier Talks

- Humoral Immunity and Antibodies – Paul Sondel
- Monoclonal Antibodies in Cancer Therapy – Ralph Schwall

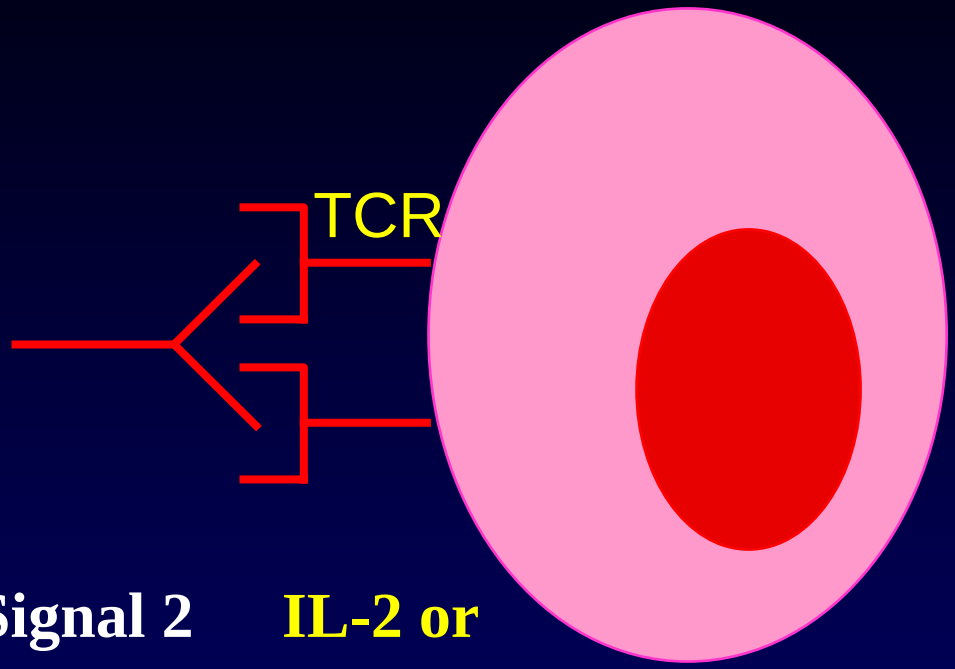
Later Talks

- Cytokines for Cancer Therapy – Jan Dutcher
- Cellular Therapies – Robert Dillman
- Critical Factors that Limit Success –Soldano Ferrone

Activated T Cells (ATC)

Signal 1

Binding of OKT3
Activates the T cells



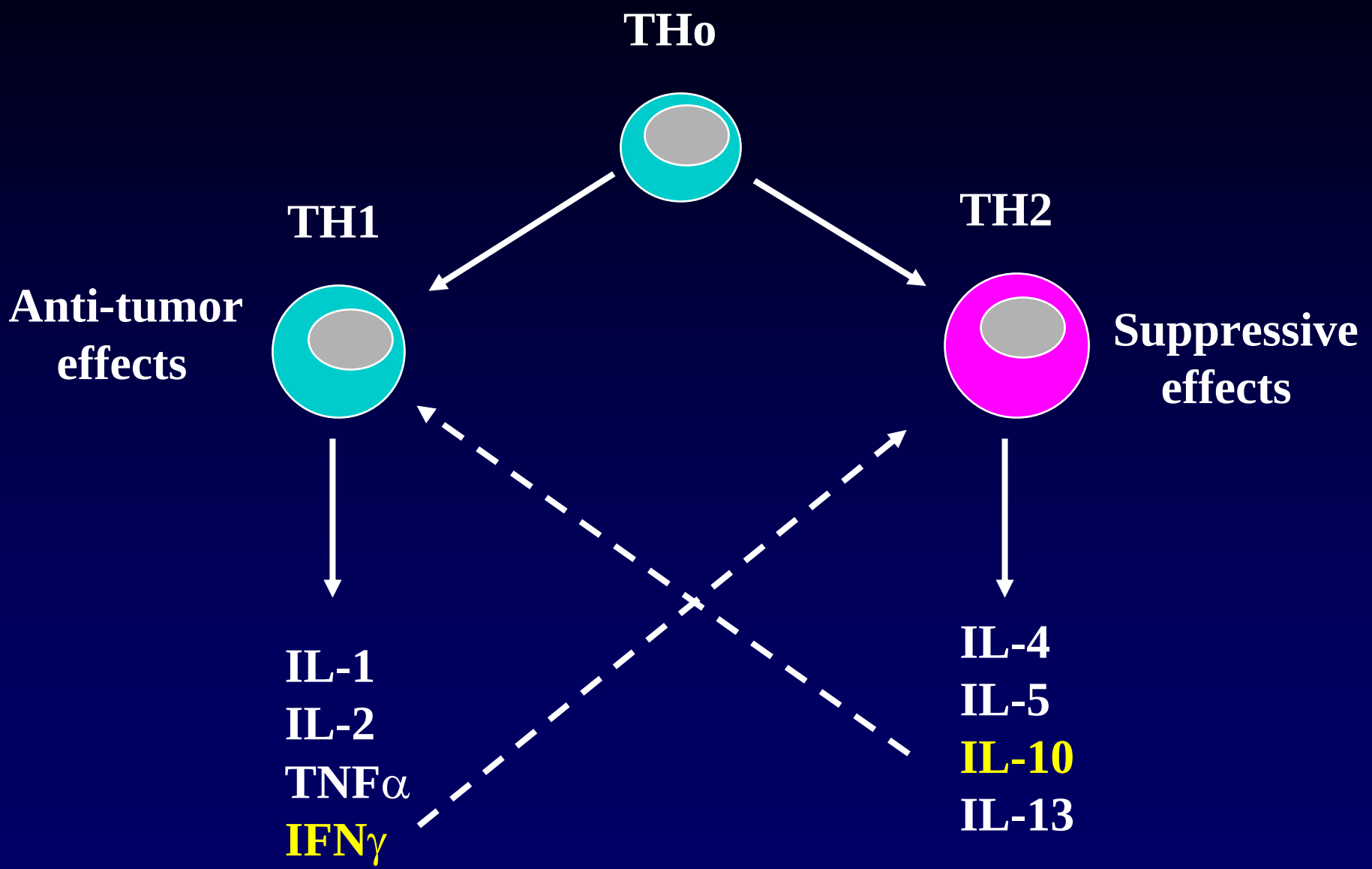
Signal 2 **IL-2 or**
Anti-CD28
Keeps T cells alive

Grow and Divide

Produce Cytokines/Chemokines

Directly Kill Tumor Cells

A Balancing Act for Anti-tumor Effects



Definitions

ATC: Activated T cells produced by anti-CD3 activation and culture in low dose IL-2 for 6-14 days.

BiAb: Consists of two mAbs produced by chemical, genetic, or hybridoma technology with 2 specificities (could be single chain fragment variable regions, scFVs).

Armed ATC: ATC with a BiAb that binds to CD3 on T cells and to a TAA on the tumor (artificial TCR).

Combination of Cellular and Humoral Therapeutic Strategy

- The specificity of monoclonal antibodies

AND

- Non MHC restricted cytotoxicity mediated by T cells, NK cells, or other effector cells

Preclinical Studies Using BiAbs

mAb	Target
Anti-Tenascin	Glioma
Anti-Glioma	Human Glioma
Anti-CD13	AML
Anti-MUC1	Bile Duct CA
Anti-EpCAM	Epithelial Cell Adhesion on AdenoCA
OC/TR	Folate Receptor on ovarian CA
Anti-kDal K29	Renal cell carcinoma
Anti-G250	Renal cell carcinoma
OKT9	Anti-transferrin receptor
Anti-AMOC-31	40 kDa membrane glycoprotein on carcinomas

mAb	Target
Anti-idiotype	BCL1 lymphoma
Anti-CD19	Leukemic B cells
Anti-tumor (Fab)2	Retargeting TIL
Anti-CEA	Carcinoembryonic antigen
Anti-Her2	Her2 on RCC, colon, breast
Anti-CD20	NHL
Anti-PSA	Prostate specific antigen
Anti-CA19-9	Carcinomas
Anti-HLA-DR beta chains	B cells
Anti-EGFR	Glioma, neuro-blastoma, colon, pancreatic,lung

BiAb Trials

- SHR-1: Anti-CD3 x anti-CD19 quadroma for NHL; 10 mcg -5 mg. No adverse effects except thrombocytopenia.
- BIS-1: anti-CD3 x anti-EGP-2 for epithelial carcinoma-associated transmembrane glycoprotein; MTD of 5 mcg/kg; induced high levels of TNF and IFN; dyspnea, vasoconstriction and fever without anti-tumor effect.
- 2B1: anti-CD16 x anti-HER2 quadroma for Her2+ tumors; DLT were fever, chills, N/V, and leucopenia; HAMA in 14 of 15; MTD was 2.5 mg/m^2.

BiAb Trials

- HRS-3/A9: anti-FCRIII x anti-CD20 for HD of B cell malignancy; MTD not reached at 64 mg/m²/dose
- MDX-H210: anti-CD64 x anti-Her2 for breast, ovarian, prostate CA; doses ranged from1-40 mg/m² without DLT
- MDX-447: anti-CD64 x anti-EGFR for renal and head and neck cancer; Hypotension DLT, doses up to 40 mg/m²
- H22x Ki-4: anti-CD64 x anti-CD30 for Hodgkin's Disease, doses up to 20 mg/m²
- Common thread: deletion of Fc portions improved toxicity profiles

Trials Using BiAb Armed T cells

- Nitta, 1990, anti-CD3 x anti-glioma armed lymphocytes; 4 of 10 pts had tumor regression.
- Lamers, 1992, ATC armed with anti-CD3 x anti-Mov28 were used to treat ovarian Ca.
- Canevari, 1995, ATC with anti-CD3 x anti-folate receptor given intraperitoneal with IL-2 resulted in tumor regression in advanced ovarian; 7 of 26 (4 CRs, 3 PRs).
- These early studies provided the impetus to develop engineered T-bodies and molecular engineering of BiAbs for targeting TAA.

Begin with the "End" In Mind

STRATEGY:
Make T Cells better killers by redirecting or focusing their non-MHC restricted cytotoxicity on TAAs

MEANS: **Arm T cells with BiAbs directed at TAAs**

GOALS:

1. **Improve tumor lysis**
2. **Immunize the patient by inducing specific CTL and humoral anti-tumor responses.**
3. **Induce remissions with persistent anti-tumor immunity.**

Phase I/II **Studies** of Ex vivo Expanded T cells

1. Phase I up to 40 billion ATC given on days 1, 4, 7, and 11 for a total of 160 billion ATC after Cytoxan/TBI and PBSCT for hematologic malignancies without adverse effects.

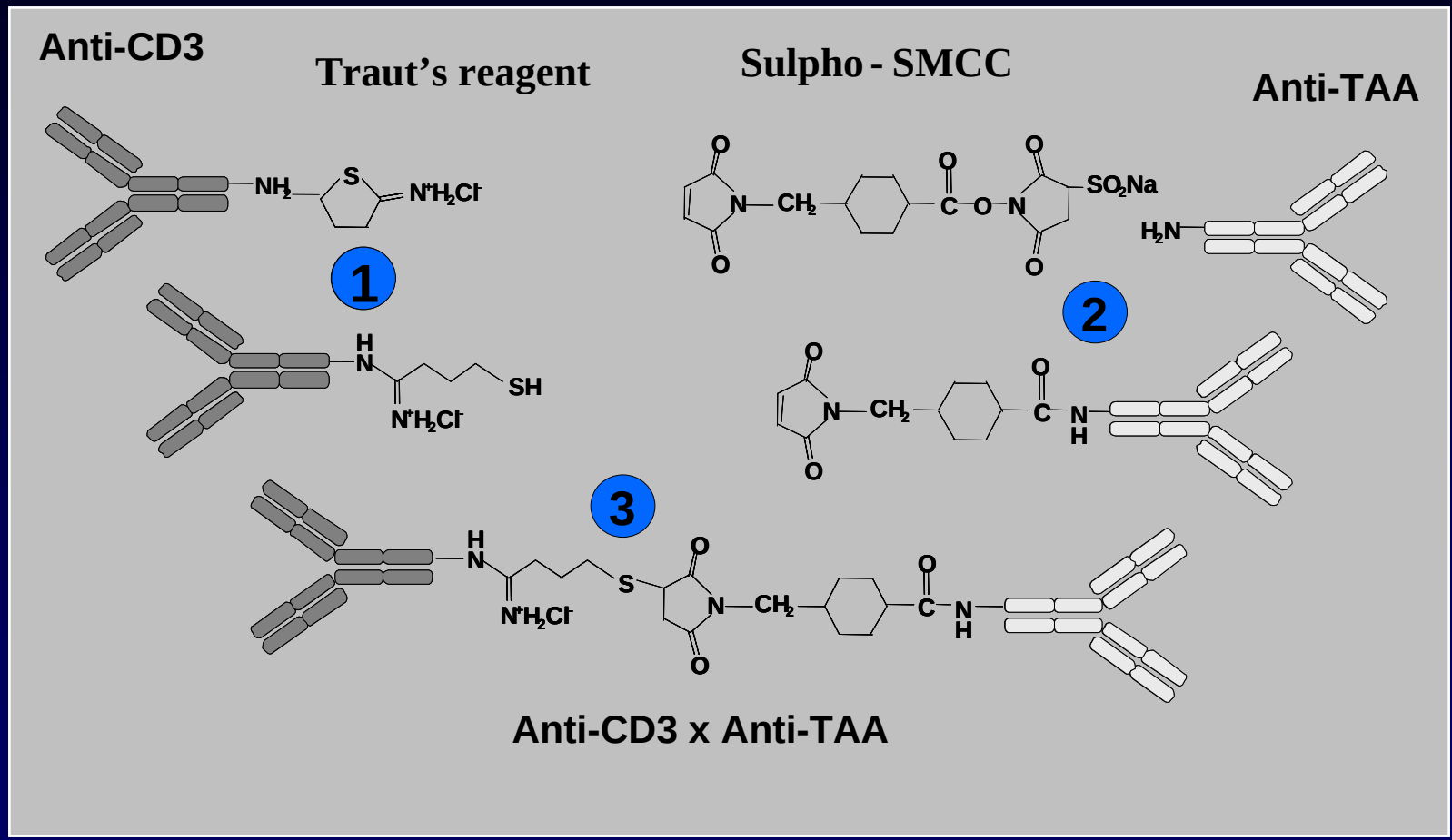
2. Phase I up to 80 billion anti-CD3/anti-CD28 coactivated T cells given in 8 doses to patients with solid tumors with IL-2. Safe with no dose limiting toxicities (J Immunotherapy 5:408, 2001) as well as with low dose Cytoxan.

3. Phase II 210 –310 billion ATC. 10 billion ATC given 3 times/wk for 3 weeks and then 20 billion/week for 6 weeks after PBSCT for stage IV breast cancer. 70% OS and 50% PFS at 32 months without regimen or cell-based adverse effects. (Autologous Blood and Marrow Transplant 10th Proc, pp95, 2001).

Development of Bispecific Antibodies

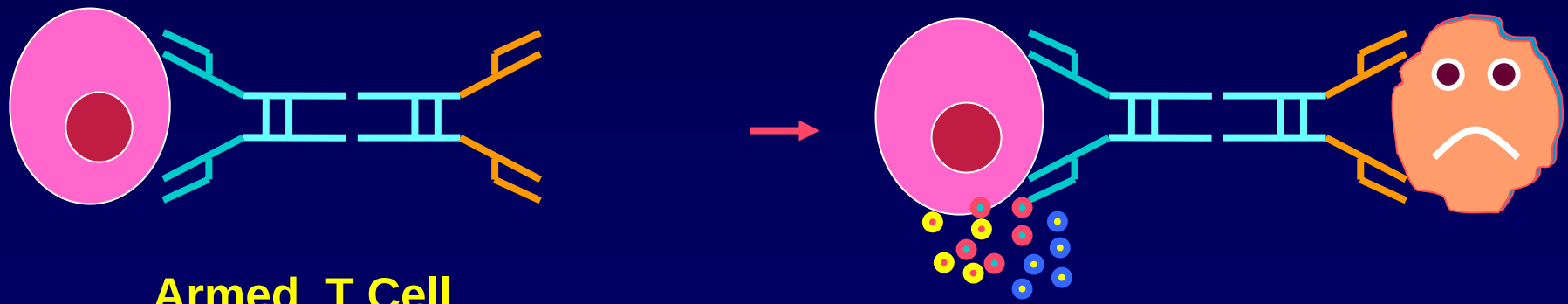
- Produced by chemical heteroconjugation of existing mAbs, recombinant DNA technology, or a combination thereof.
- A variety of design formats allow for: 1) different sizes to allow tissue penetration; 2) enhanced specificity; 3) increased affinity to effector cells.
- Applications: targeting effector cells, targeting toxins, drugs, prodrugs, enzymes, DNA, anti-vascular agents, gene therapy vectors, radionuclides, and others (use your imagination)

BiAb Production by Chemical Heterconjugation



Targeted Killing by T cells with BiAbs

Anti-CD3 Anti-TAA Anti-CD3 x Anti-TAA



Armed T Cell

T Cell

Tumor Lysis

Production of Armed T cells

PBMC from Pheresis

↓

OKT3 (20 ng/ml) + 100 IU/ml of IL-2

↓

ATC are split every other day

↓

Harvest, Arm with BiAb and Cryopreserve after 10-14d

↓

Quality Control (Bacteria, fungal, and Mycoplasma stain) 7 days

↓

Testing for cytotoxicity and cytokine production

Characteristics of Armed ATC

1. Exhibit non-MHC restricted cytotoxicity (“promiscuous killers”).

2. Secrete IL-2, IFNγ, TNFα, GM-CSF, MIP-1, and RANTES after antibody receptor binding.

3. >95% CD3+ cells, 60-80% CD8 cells, 20-40% CD4 cells, and <5% CD56+ cells.

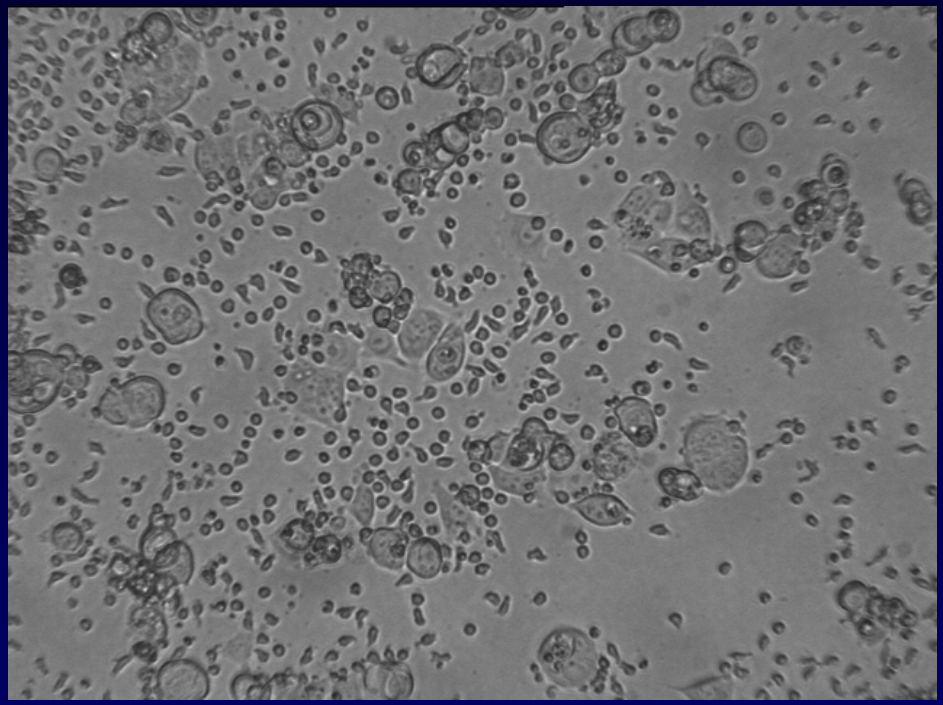
4. Patient CD3 cells expand up to 30 fold in 14 days.

Preclinical Questions

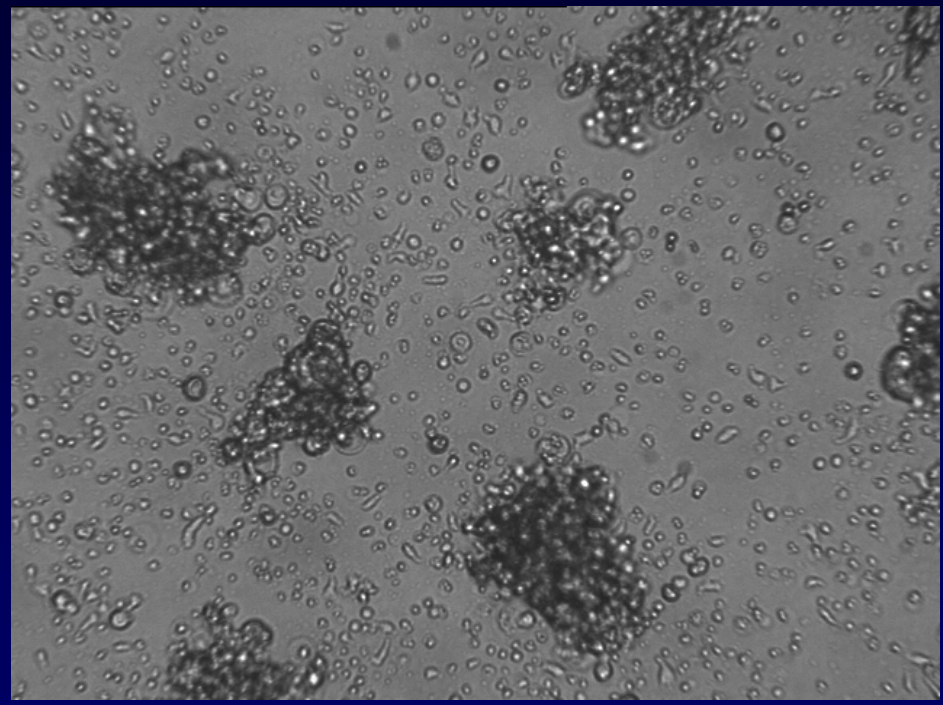
- How long will the BiAb remain on ATC?
- How long will armed ATC kill?
- Will binding to tumor trigger cytokine secretion?
- How many times will armed ATC kill?
- How long can armed ATC be detected in patients?

Targeting and Killing

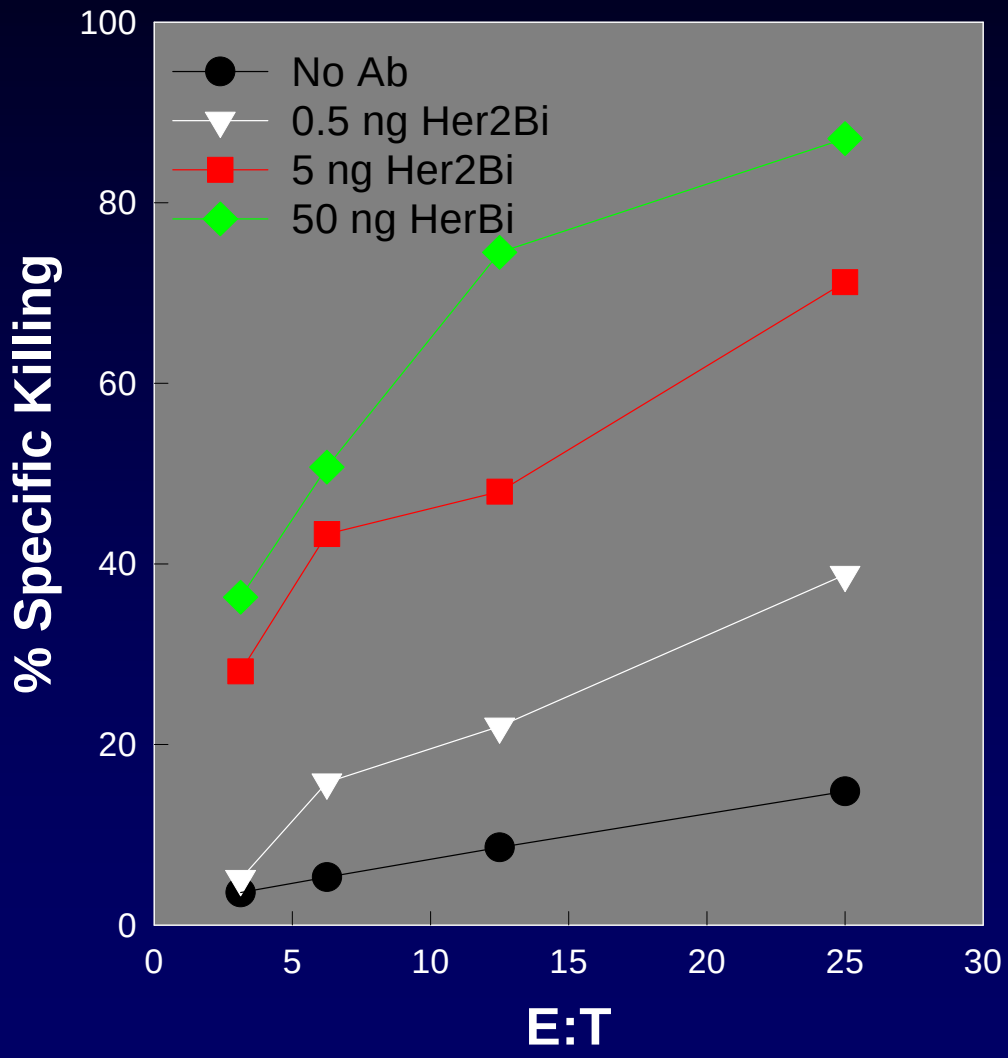
Unarmed



Armed

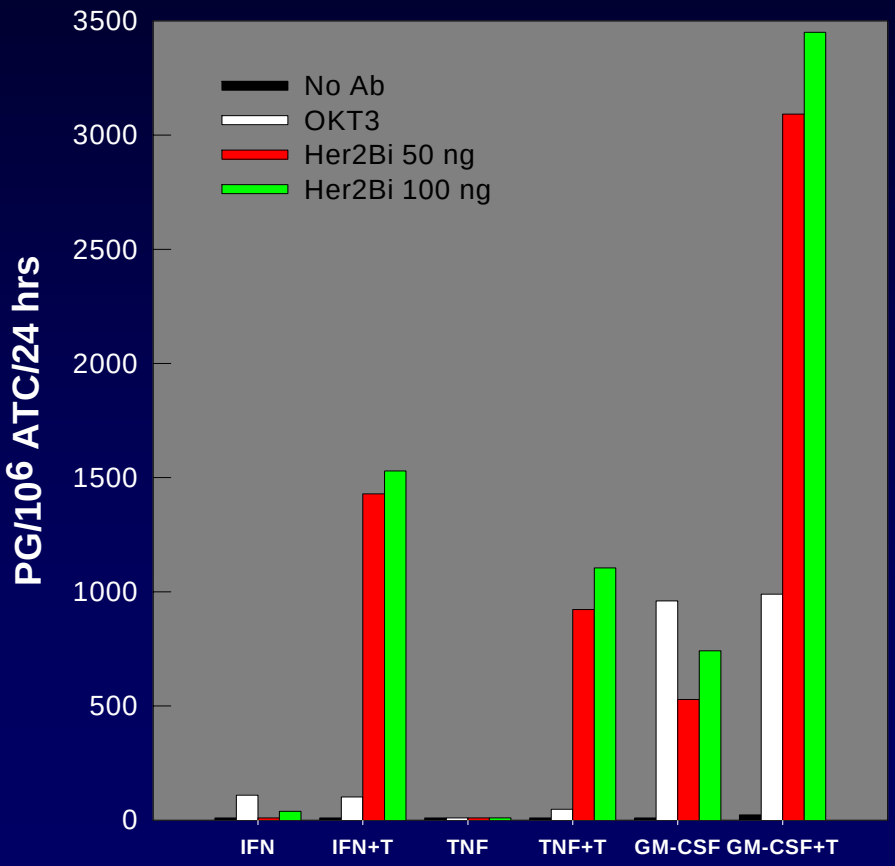


Killing of MCF-7 Cells by Armed T Cells

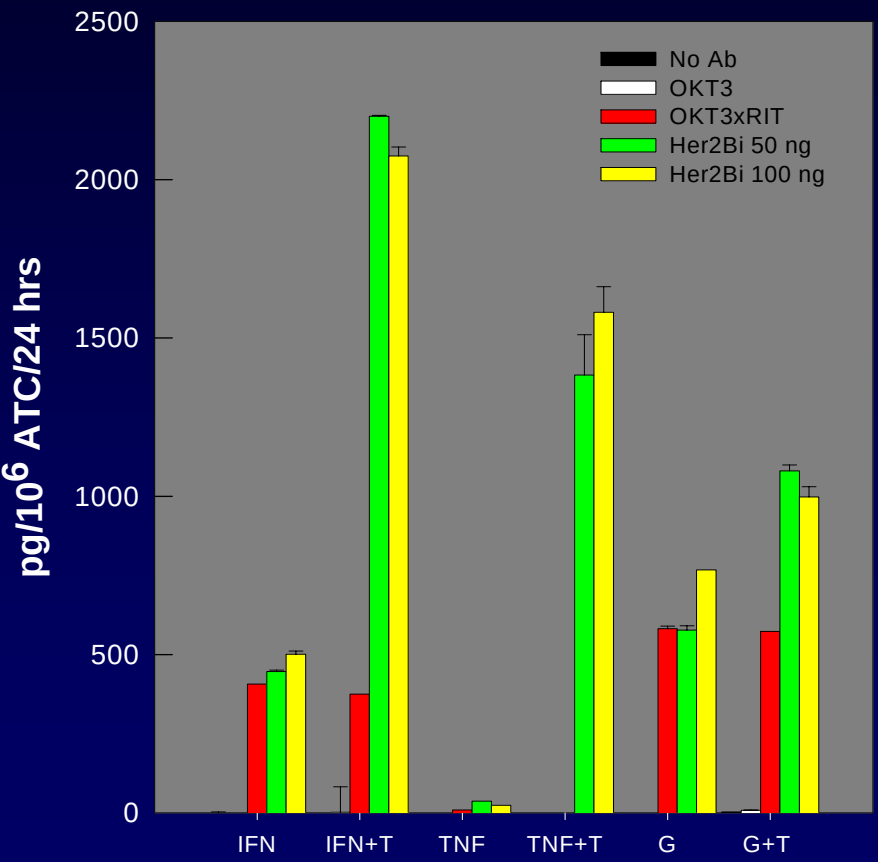


Cytokine Production by Armed T Cells Exposed to SK-BR3

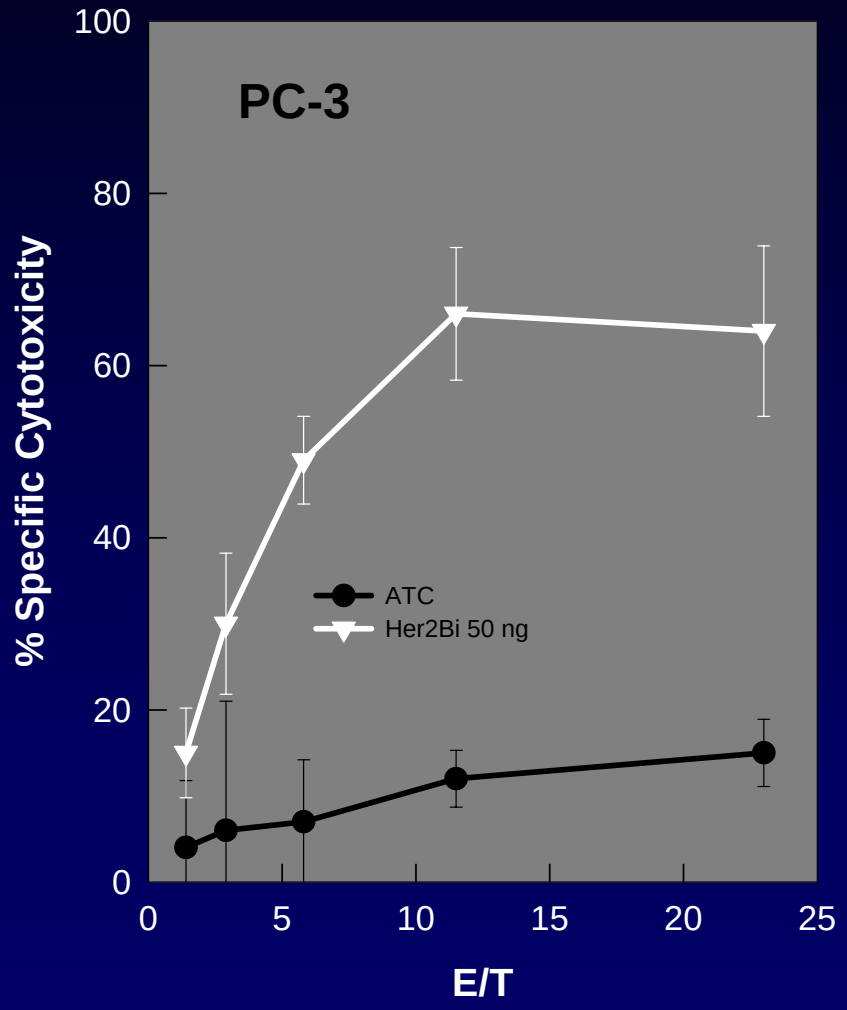
Normal

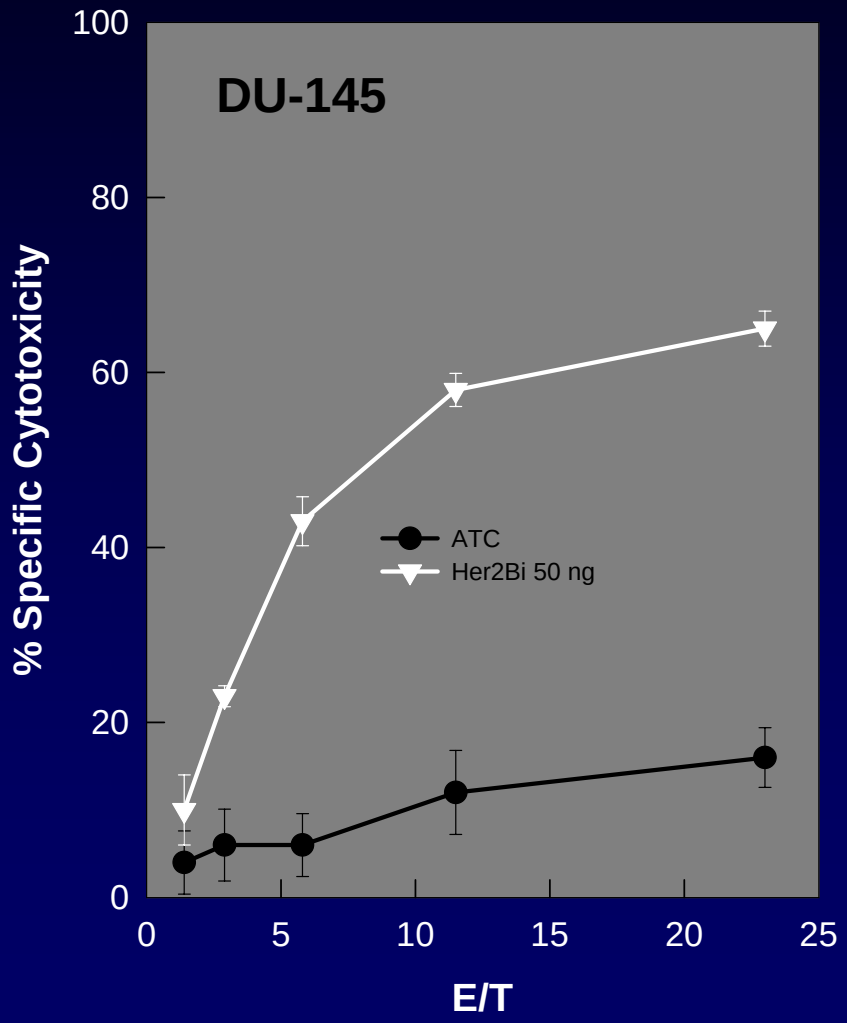


Patient

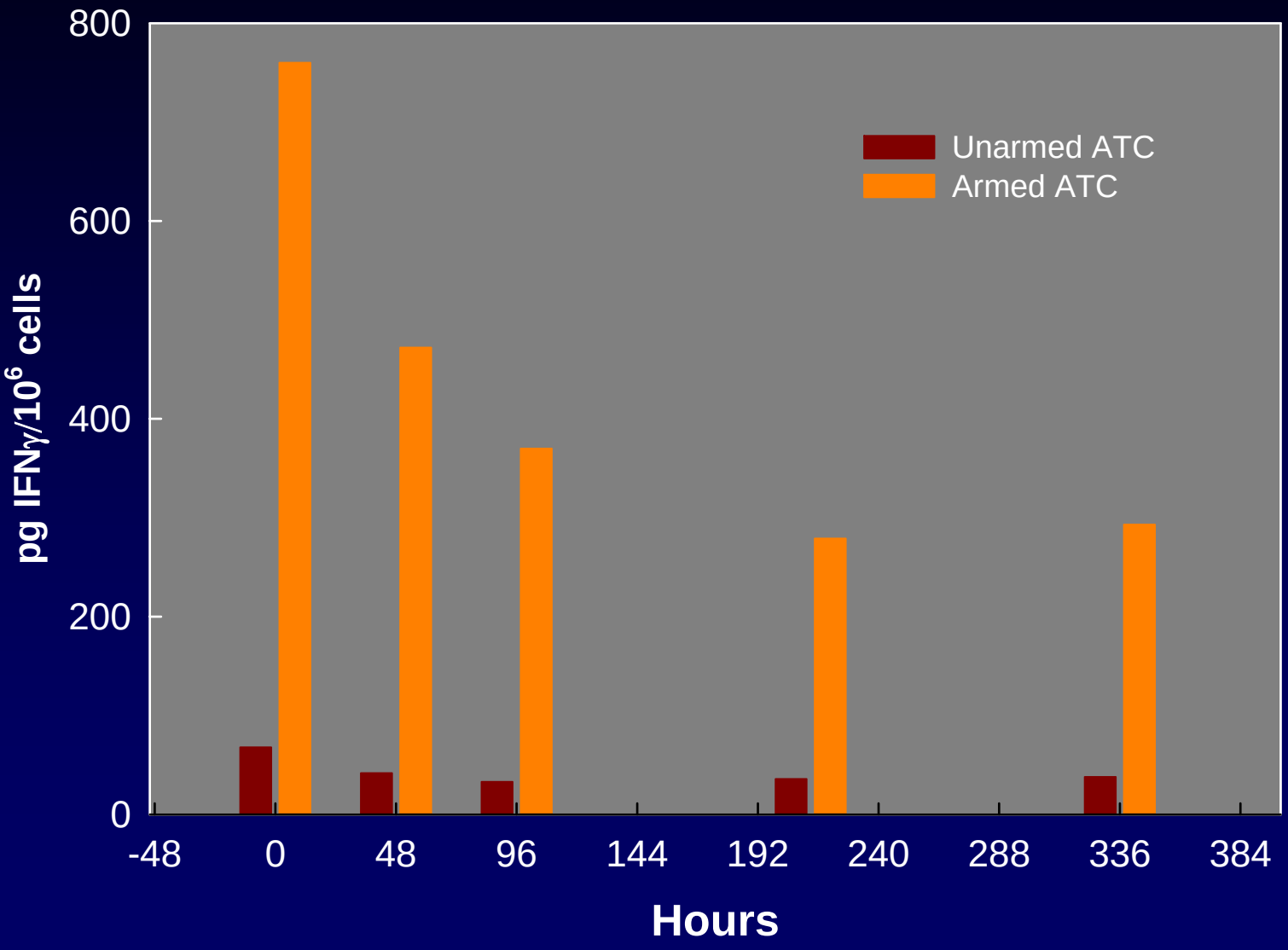


Cytotoxicity **Directed** at Prostate Cancer Lines

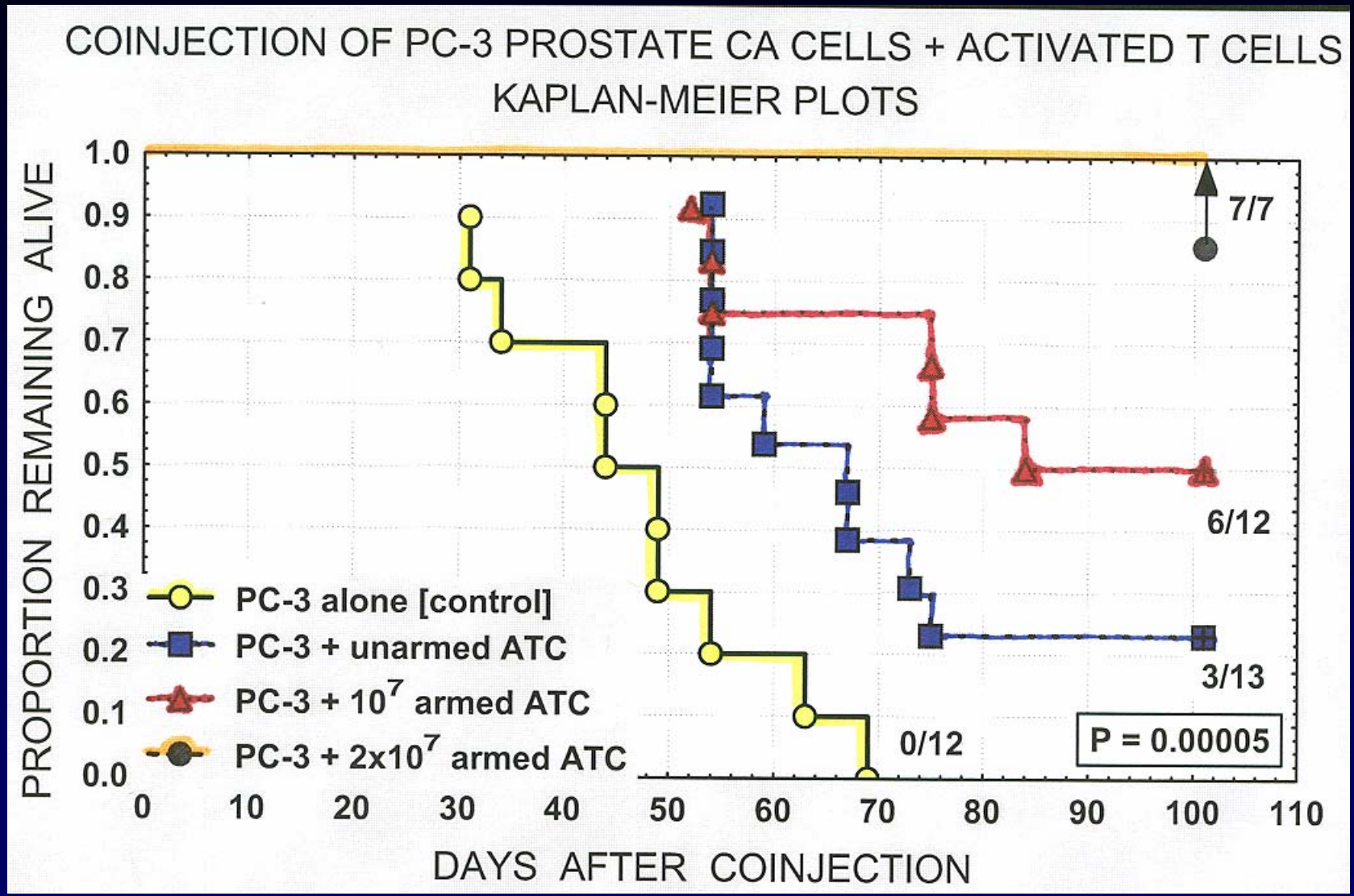




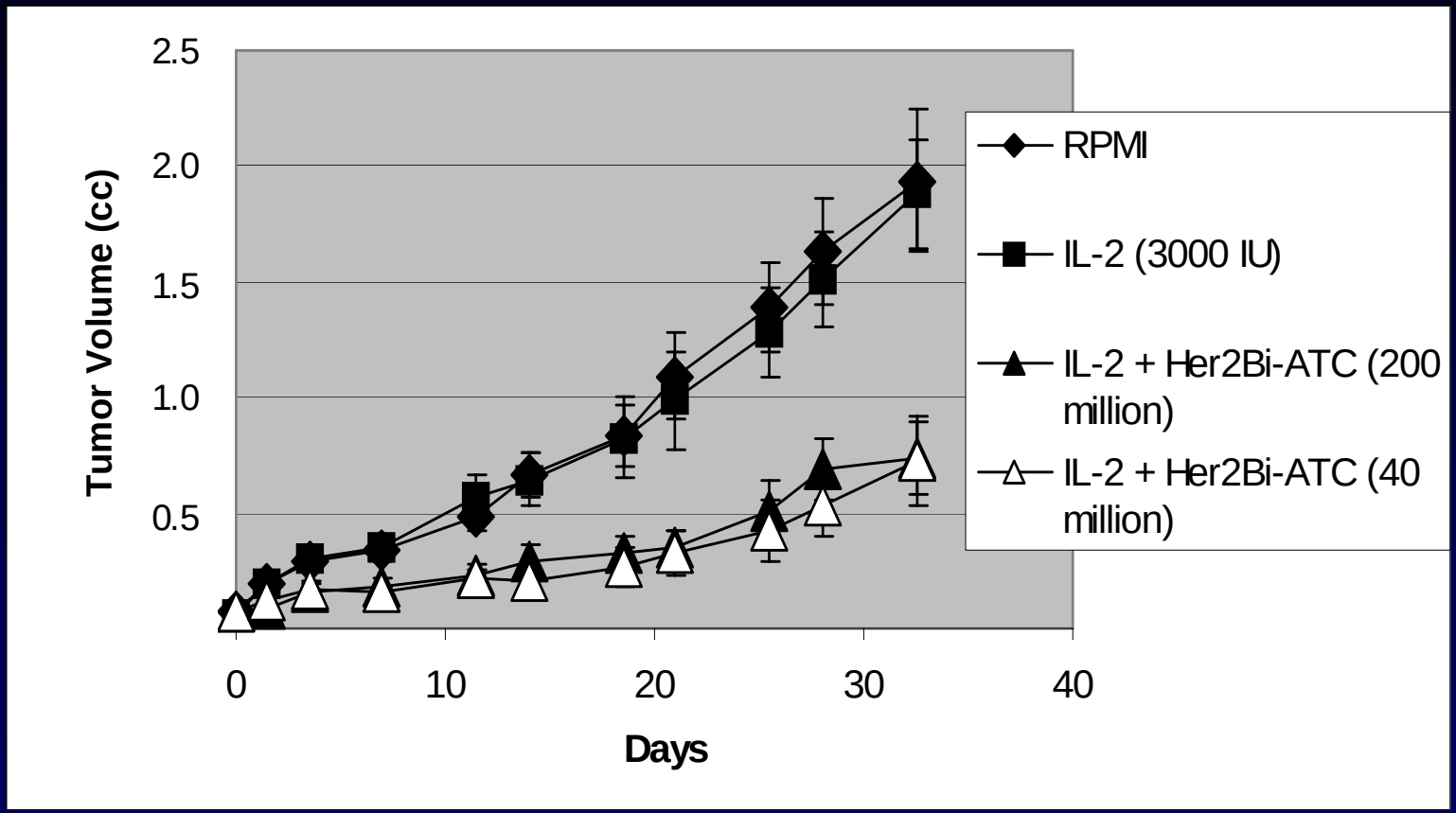
IFNγ Secretion upon Repeated SK-BR-3 Restimulation



Prevention of Prostate Cancer

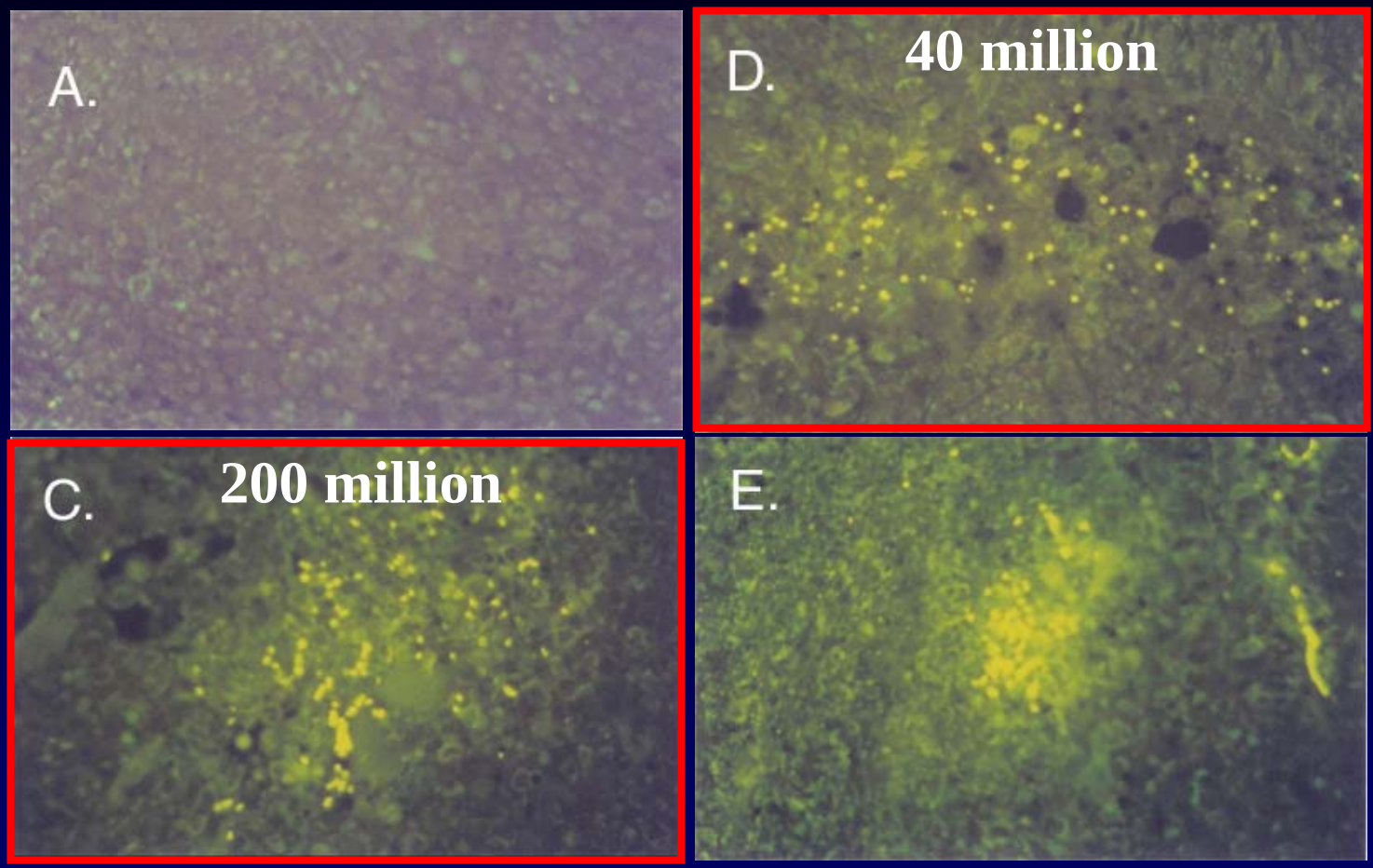


IV Treatment Delays PC-3 Xenografts



PC-3 tumor cells (10^7) were implanted SC in flanks of SCID-Beige mice. 7 days later when tumors were ~0.05 cc, treatments were started once/week x 4 weeks and tumor growth monitored. Results are from 2 experiments (n=10 mice/group).

Trafficking of Her2Bi Armed ATC in Beige/SCID



A RPMI/ IL-2 IV; C) IL-2 + armed ATC (2 x 10^8 cells) IV; D) IL-2 + armed-ATC (4 x 10^7 cells) IV; or E) IL-2 (3000 IU) + armed ATC (2 x 10^8 cells)IT. Tumors were excised 18 hr after treatment, formalin fixed, paraffin embedded, sectioned, and stained for human CD3+ cells

Immunotherapy Approaches

Cells	CMV CTL to Prevent Infection	EBV for LPD	MM Specific CTL after Chemo	DLI after Allo-BMT	ATC after HDC+PBSCT for BrCa	Armed ATC
Type	Specific	Specific	Specific	Polyclonal	Polyclonal	Specific
Dose	**10^6/kg**	**~.5x10^9/kg**	**10^9/kg**	**10^8/kg**	**3 x 10^9/kg**	**4 x 10^9/kg (0.5x 10^9/kg)**
Effect	**Prevents CMV Pneumonia**	**Treatment of LPD**	**Induce CR in MM**	**Induce CRs in CML>AML>ALL**	**Improve PFS?**	**Decrease Bone Pain, PSA, CA 27-29**
Target	**Viral**	**EBV LPD**	**Solid tumor**	**Liquid tumors**	**Solid tumor**	**Solid tumors**

Protocols: FDA and IRB Approved

Breast Cancer

- RWH #356-46: TAC + Armed ATC for Stage II-III BrCa
- RWH #351-46: Armed ATC for Stage IV BrCa (NCI-R01 funded)

Hormone Refractory Prostate Cancer

- RWH #355-46: Armed ATC for HRPC

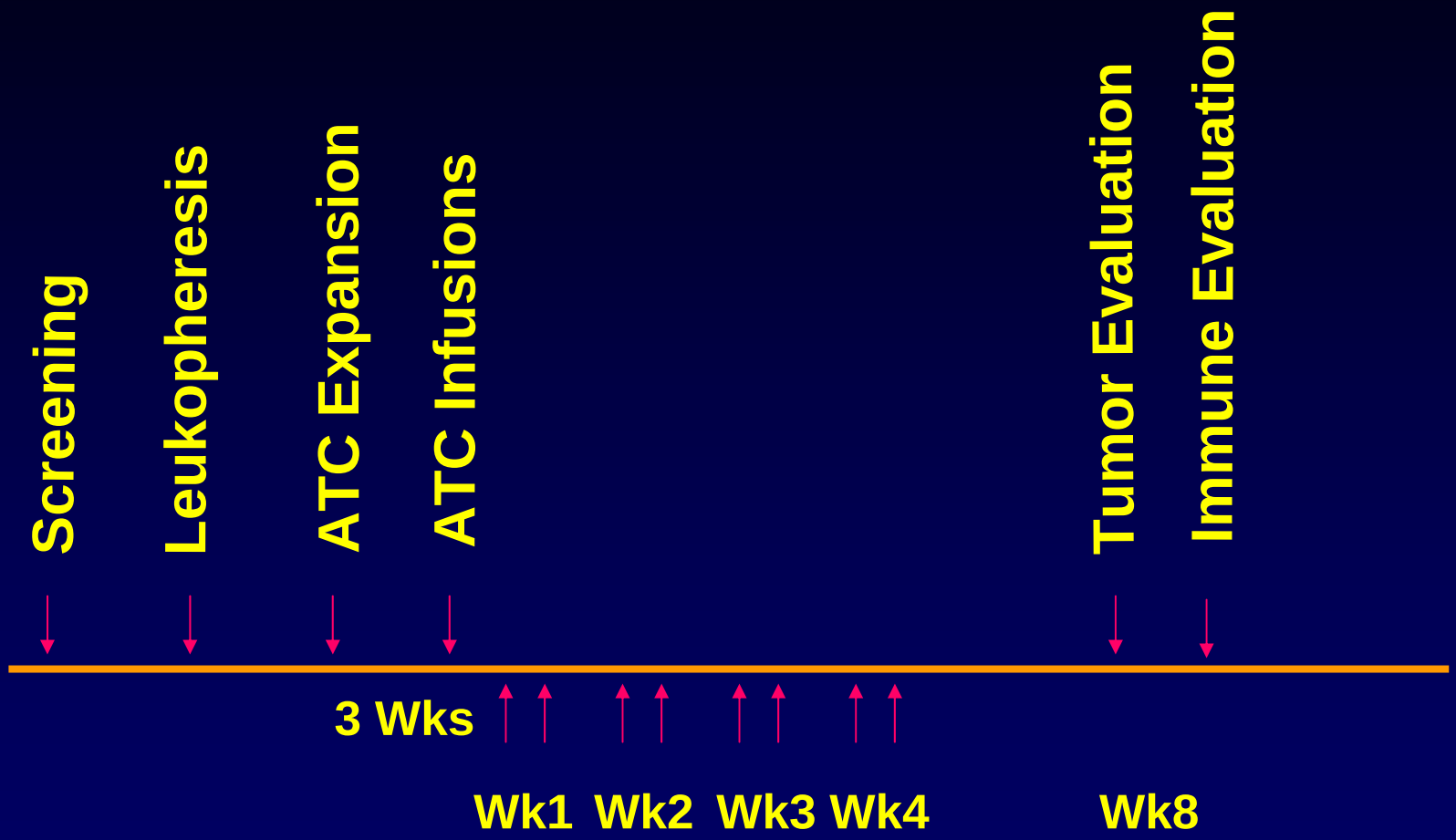
Eligibility:

- Metastatic, measurable, or evaluable sites
- Phase I: Her2/*neu* positive or negative
- Phase II: Her2/*neu* positive
- No active cardiac disease, ECOG PS 0-2, life expectancy >3 months

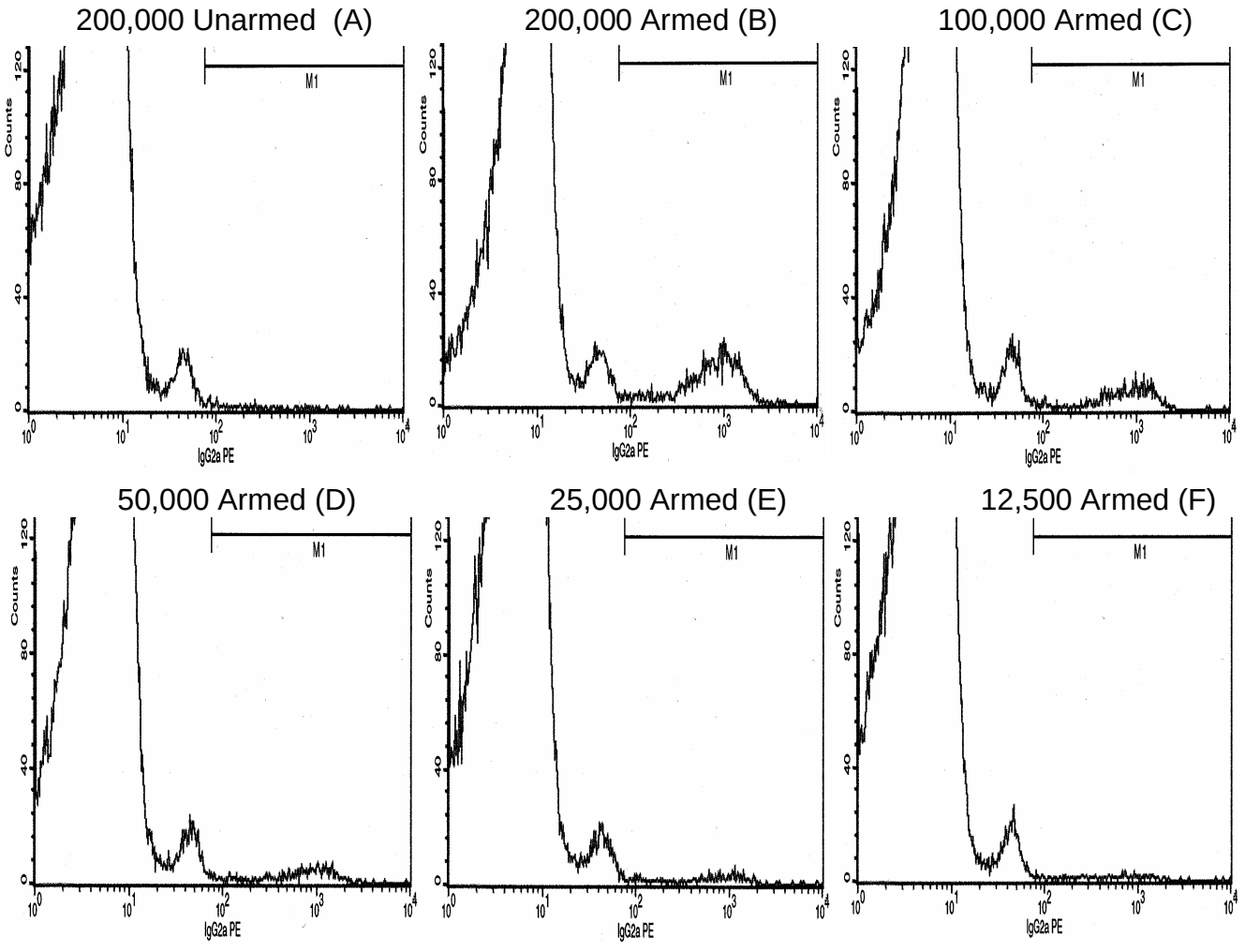
Lymphoma

- RWH #394-46 : Armed ATC targeting CD20 lymphomas after PBSCT
  FDA approved 11/02/04 and (Leukemia & Lymphoma Society funded)

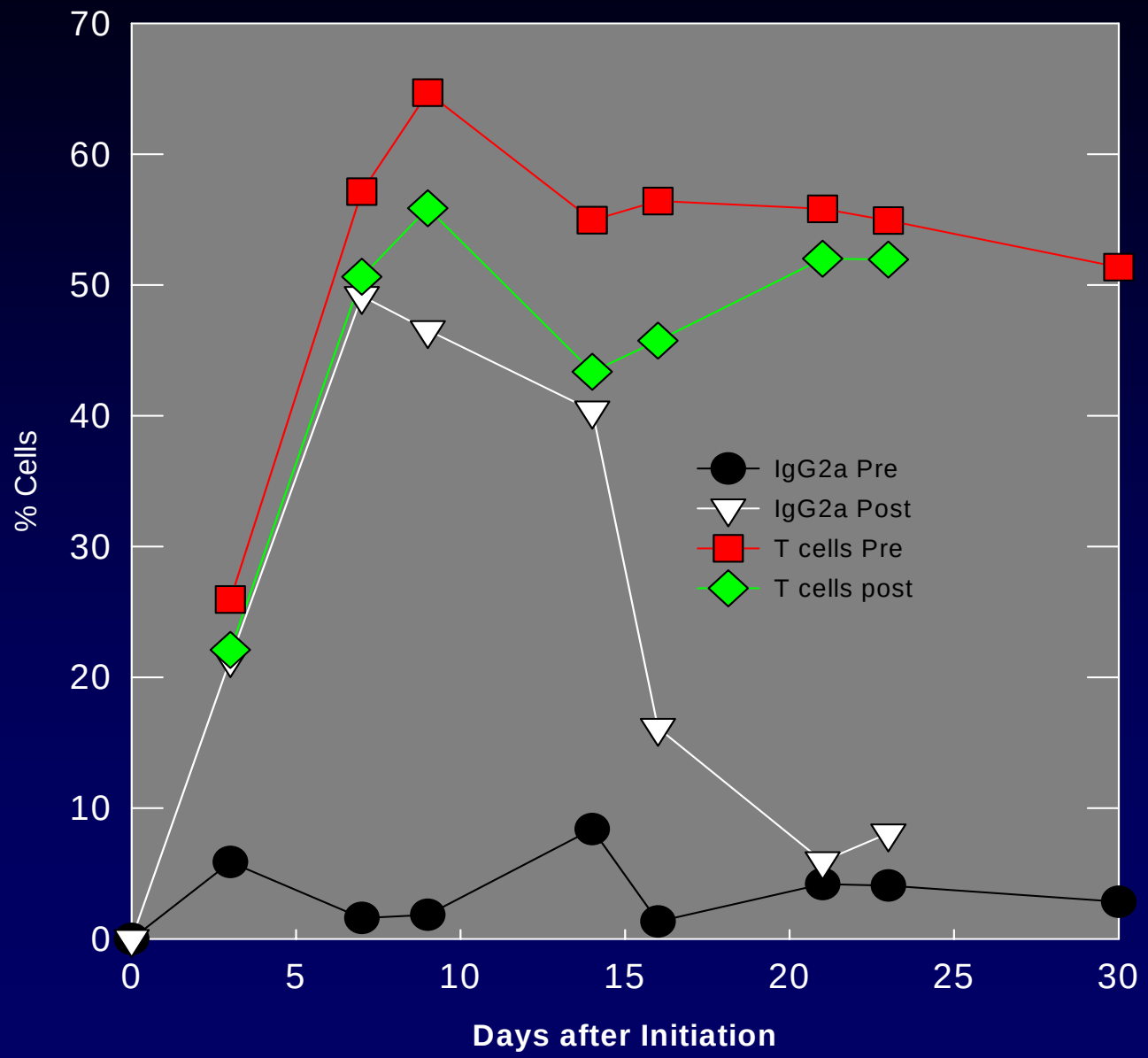
Treatment Schema for Breast and Prostate



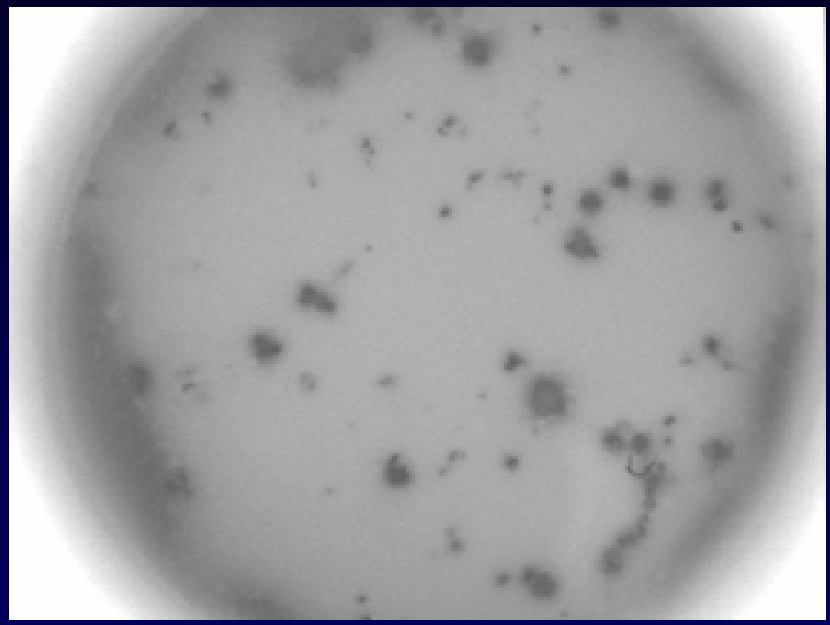
Whole Blood Spiked with HER2Bi ATC

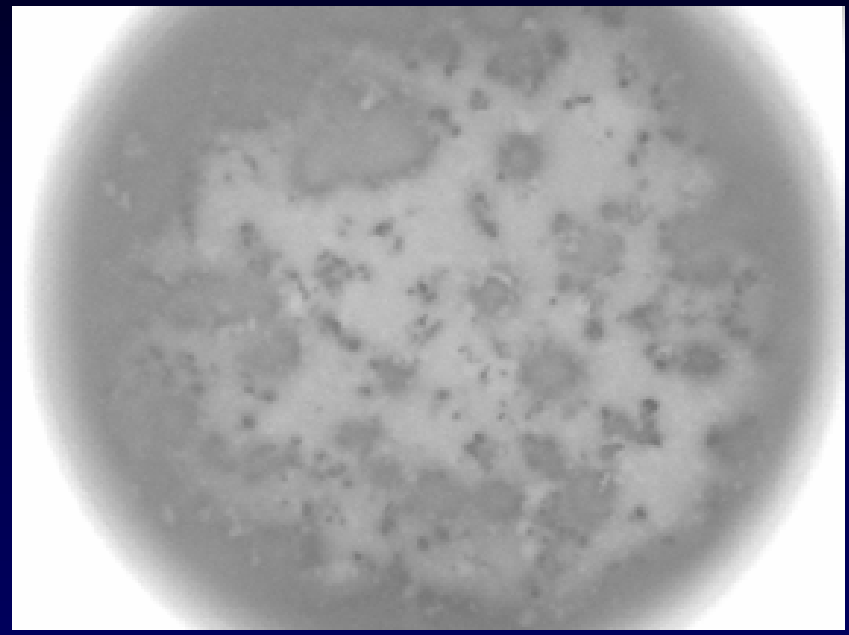


Detection of Armed ATC in Blood



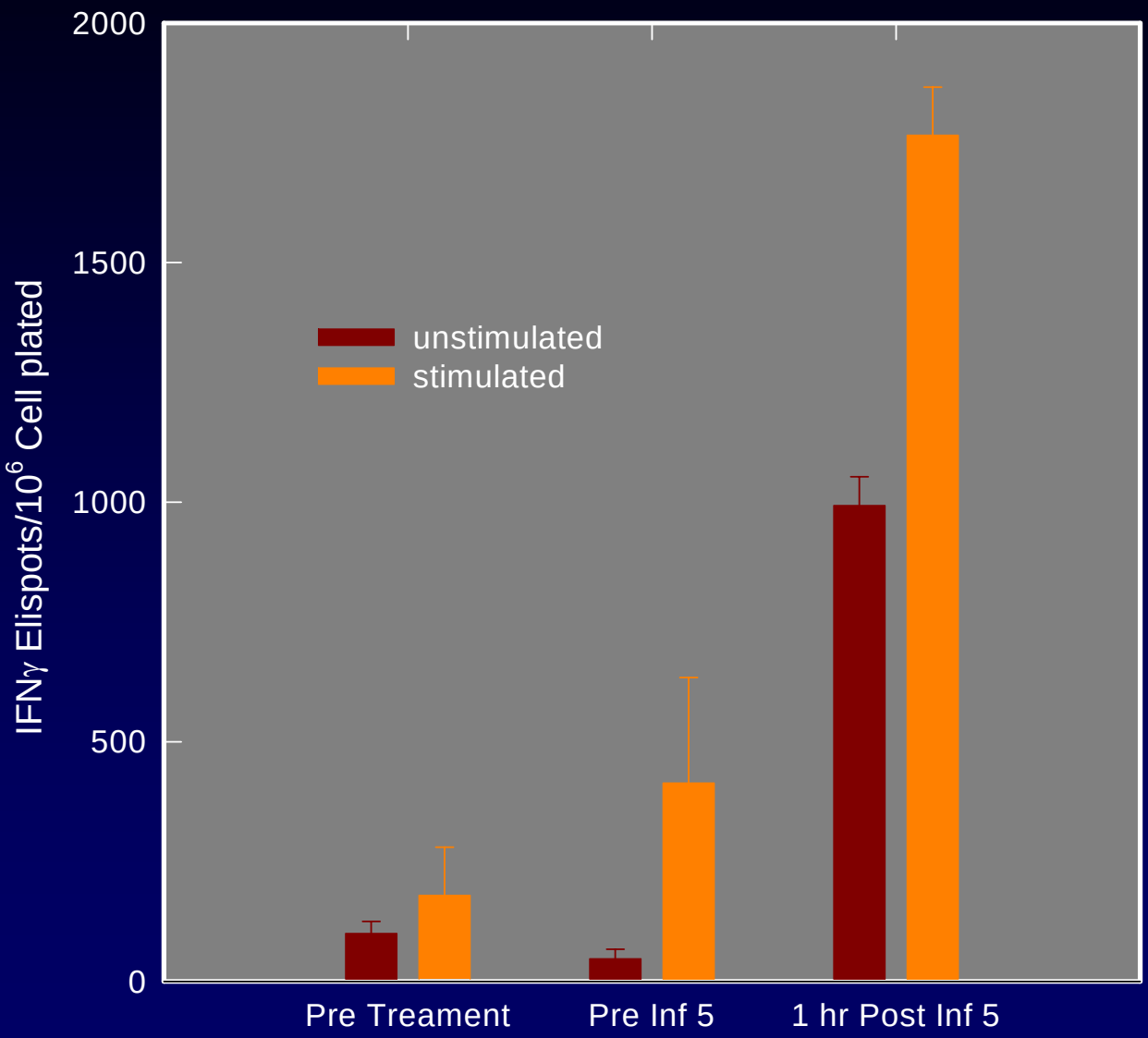
Pre and Post EliSpots



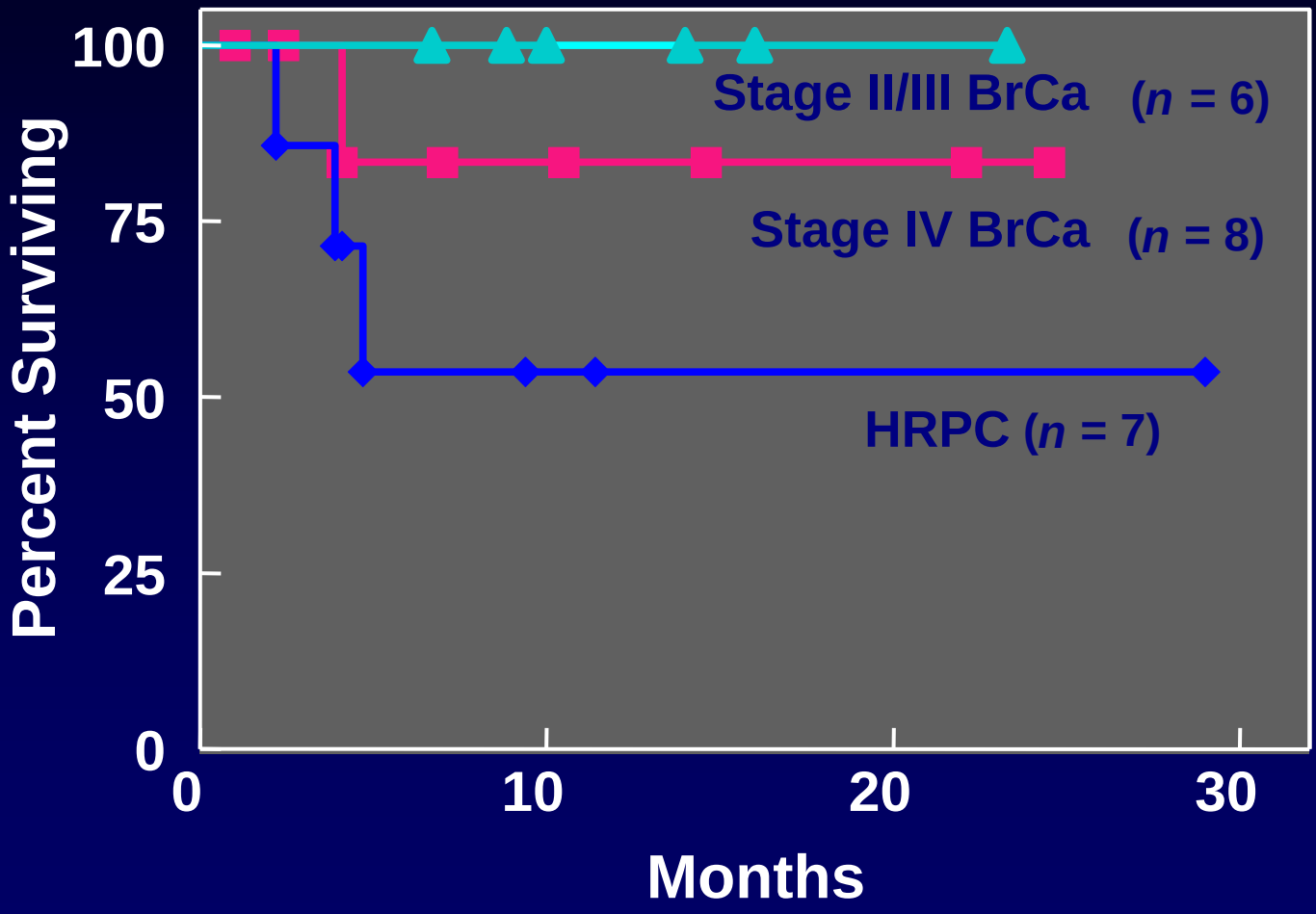


IFN-γ EliSpots with PBMC isolated from a patient before and after the 4th infusion of Her2Bi-armed ATC (5 x 10^9). Her2/*neu*-specific IFN-☎ secretion by T cells was measured by exposing 10^5 PBMC to SK-BR-3 cells at an E:T of 10 for 2 h at 37°C and then the PBMC to an EliSpot plate coated with anti-IFN-γ.

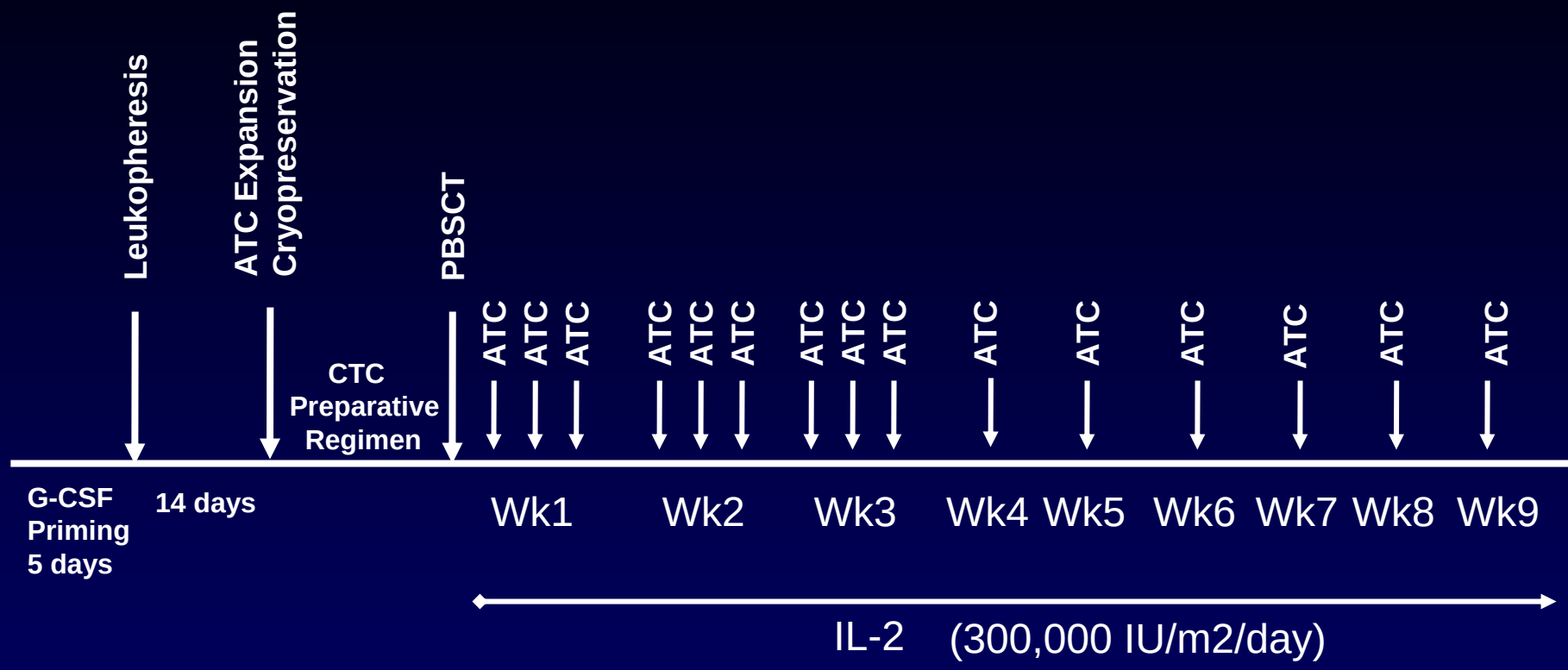
EliSpots from Stage IV BrCa Pt



Overall Survival in Phase I Trials with Her2Bi-armed ATC



RWH# 04-394-46 Infusion of ATC Armed With CD20Bi for CD20+ NHL



Dose Level 1: 5 billion/infusion Total 75 billion
Dose Level 2: 10 billion/infusion Total 150 billion
Dose Level 3: 15 billion/infusion Total 225 billion
Dose Level 4: 20 billion/infusion Total 300 billion

Armed ATC

- Grow and divide after engaging and killing the tumor
- Secrete chemokines and cytokines multiple times
- Bind and kill tumors cells multiple times
- Survive in vivo > 3 weeks
- Develop into Ag-specific CTL over 2 weeks in culture
- Patients infused with armed ATC develop levels of cytokines during their infusion
- PBMC from patients develop cytotoxicity that persists up to a month after the last infusion

Summary for Armed ATC

1. Armed ATC kill multiple times.

2. Armed ATC proliferate after engaging tumor and do not undergo apoptosis via Fas/FasL or ACID.

3. Large numbers (320+ billion) of armed ATC can be produced in 2 weeks whereas cloned CTL are time consuming requiring a customized effort.

Summary for Armed ATC

4. **Armed ATC may develop into Ag-specific CTL directed at other TAA AND induce endogenous T cells to become cytotoxic.**

5. **“Multiple infusional vaccinations” may immunize patients to their autologous tumor.**

6. **Significant amounts of cytokines are found in patient serum during and after infusions with a Th1 profile.**

7. **There is a strong suggestion that overall survival for metastatic breast and HRPC patients is improved even with small numbers of patients.**

Platform Technology

OKT3 + Anti-Her2/*neu*

Breast

Prostate

Eight infusions of armed ATC

OKT3 + Anti-CD20

Lymphoma

ALL?

CLL?

PBSCT + Armed ATC

OKT3 + Anti-EGFR

Colon

Pancreatic

Lung

Glioblastoma

Neuroblastoma

Concepts/Principles

- Bispecific antibodies can be used to target effector cells to tumors.
- The non-MHC restricted cytotoxicity can be redirected with BiAbs.
- The targeted cell therapy can be used in combination with cytokine, chemotherapy, or stem cell transplant strategies that make immune space and reduce tumor burdens.
- The platform allows flexibility for targeting different TAAs by switching BiAbs

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Facilities:

1. Specialized ultra-clean rooms that are FDA approved for producing T cells and bispecific antibodies for clinical trials.
2. Seamless clinical coordination between institutions/practices
3. Immunotherapy clinic that is staffed with experienced nurses.