

Targeting Breast Cancer Through the Immune System

Michael S. Sabel, MD

Division of Surgical Oncology

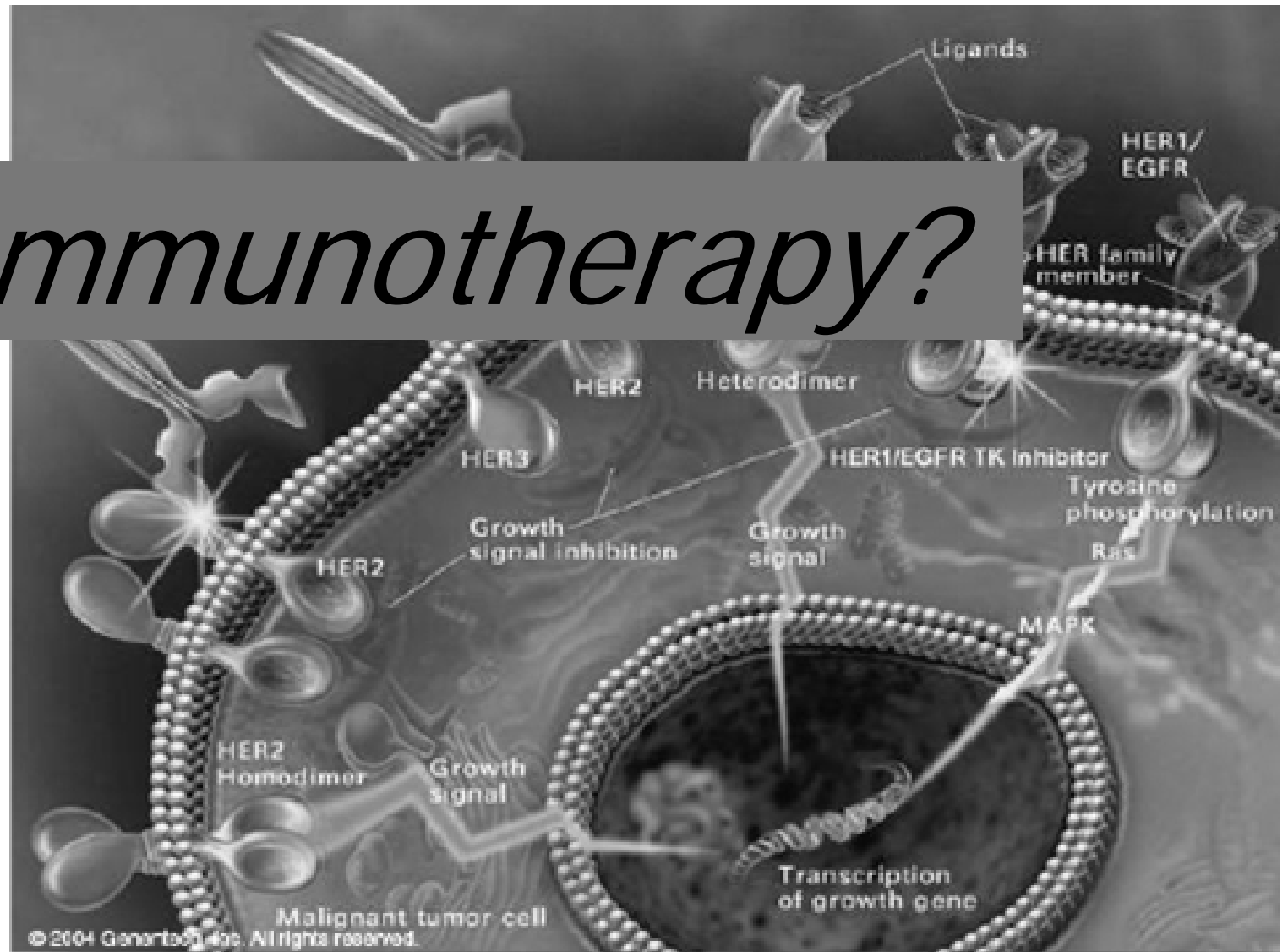
University of Michigan

Comprehensive Cancer Center

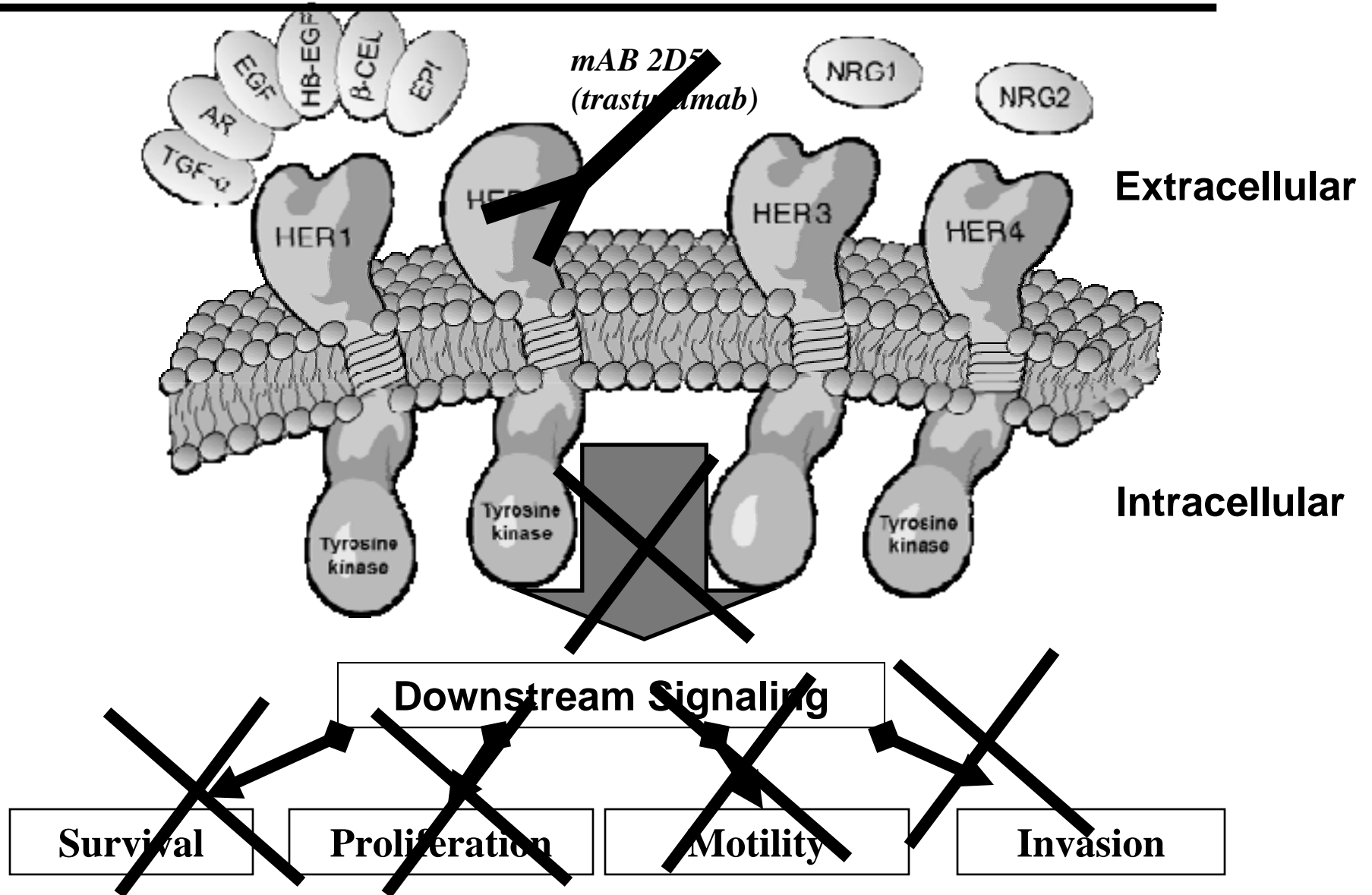


Trastuzumab (Herceptin®)

Immunotherapy?

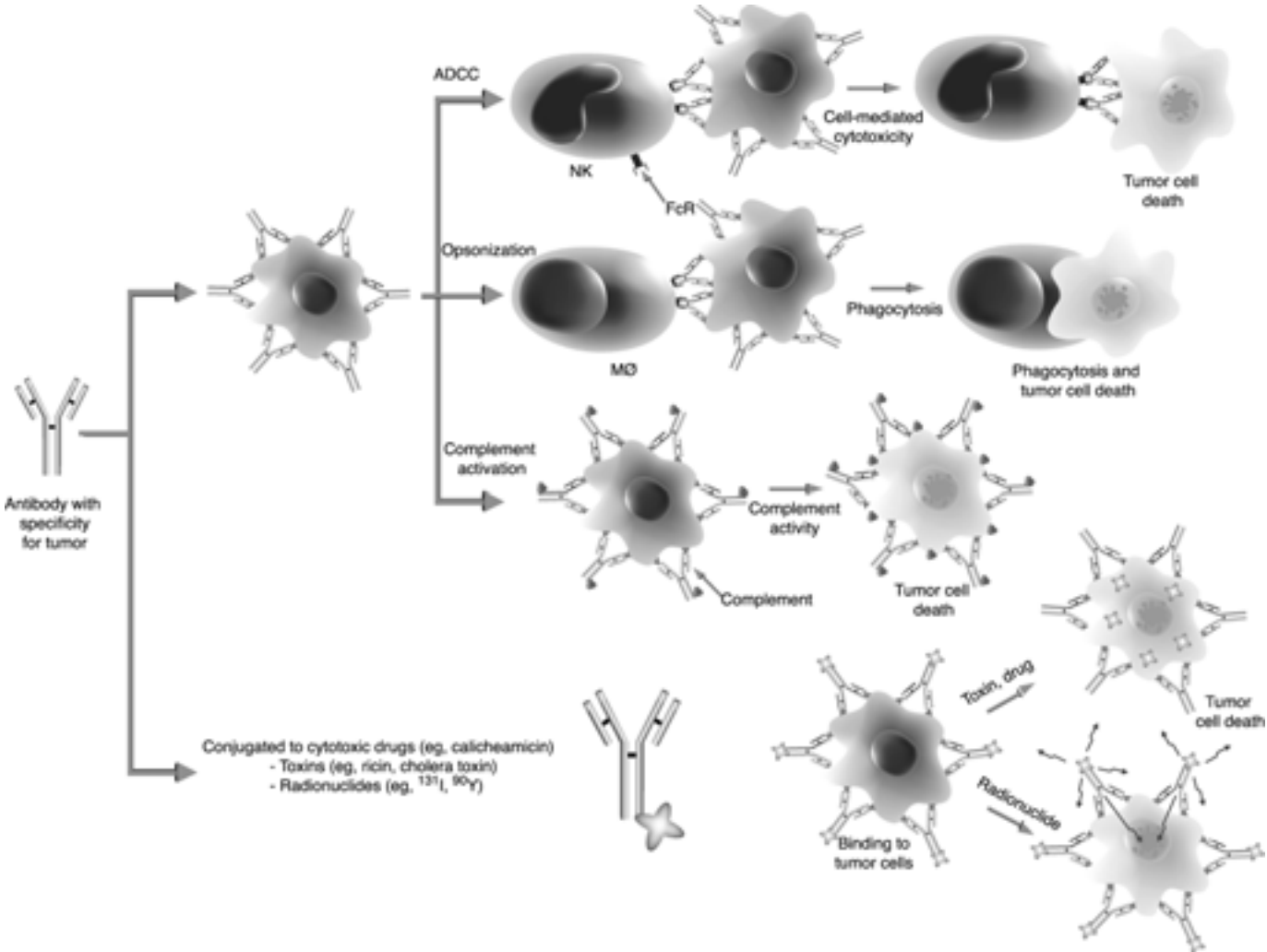


Trastuzumab (Herceptin®)



Adapted from Tzahar and Yarden. Biochim Biophys Acta. 1998;1377:M25.

Direct and Indirect Mechanisms Through Which Antitumor Antibody Can Mediate Tumor Cell Killing Directly



Passive Immunotherapy Using Monoclonal Antibodies

Efficacy of Anti-Cancer mAbs

Antibody Property	Clinically Effective	Clinically Ineffective
Signal Perturbation	Trastuzumab Rituximab Cetuximab Bevacizumab	?
No signal perturbation	Alemtuzumab	>100 antibodies

Adams GP & Weiner LM. Monoclonal antibody therapy of cancer. Nature Biotechnology 2005;23(9):1147.

DF3 targeting MUC-1

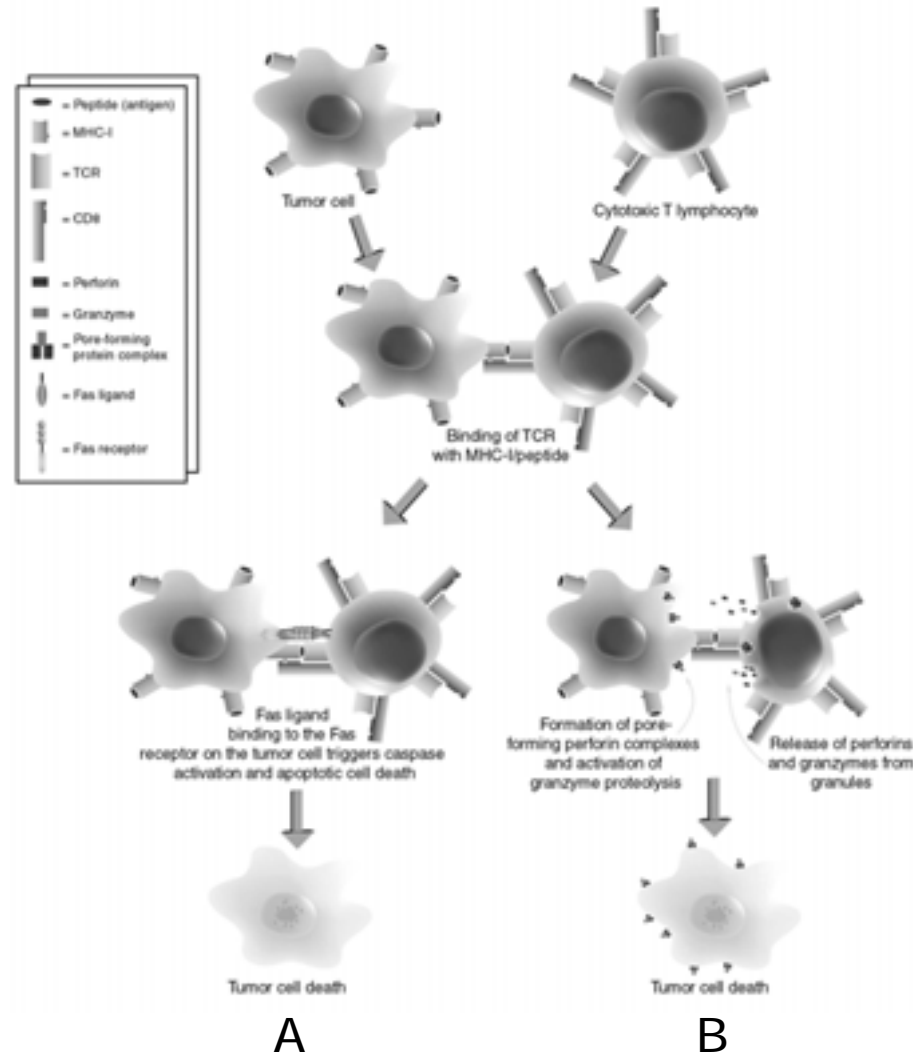
Edrocolomab targeting EpCAM



Passive Immunotherapy
Using Cellular Therapy

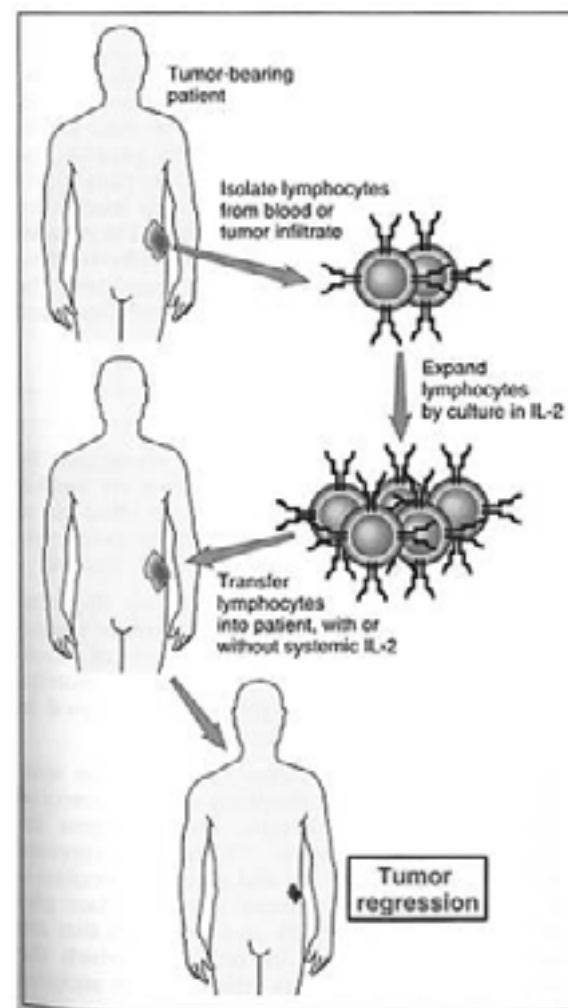
Adoptive
Immunotherapy

Effector Mechanisms of Tumor Cell Killing by Cytotoxic T Cells (CTLs)



Passive Cellular Immunotherapy In Breast Cancer

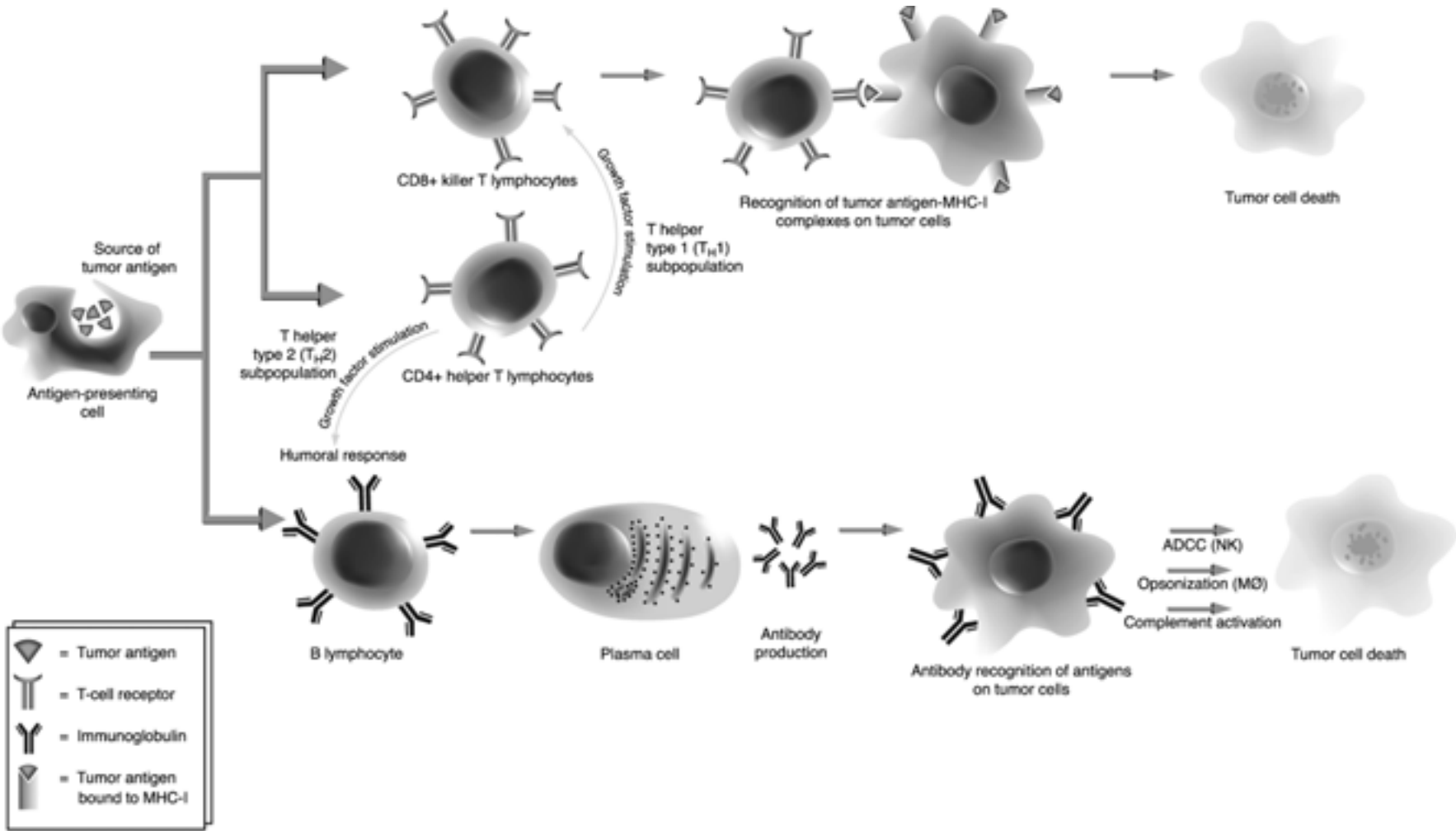
- Antigen Specific T-cells
(blood or TIL)
 - difficult to obtain TIL from breast tumors
 - difficult to expand autologous breast cancer cell lines for targets of T-cell lytic activity
 - location of relapse (brain, bone) difficult to obtain adequate tissue.
- Other Possibilities
 - Tumor Draining Lymph Nodes (TDLN)
 - Vaccine Draining Lymph Nodes (VDLN)



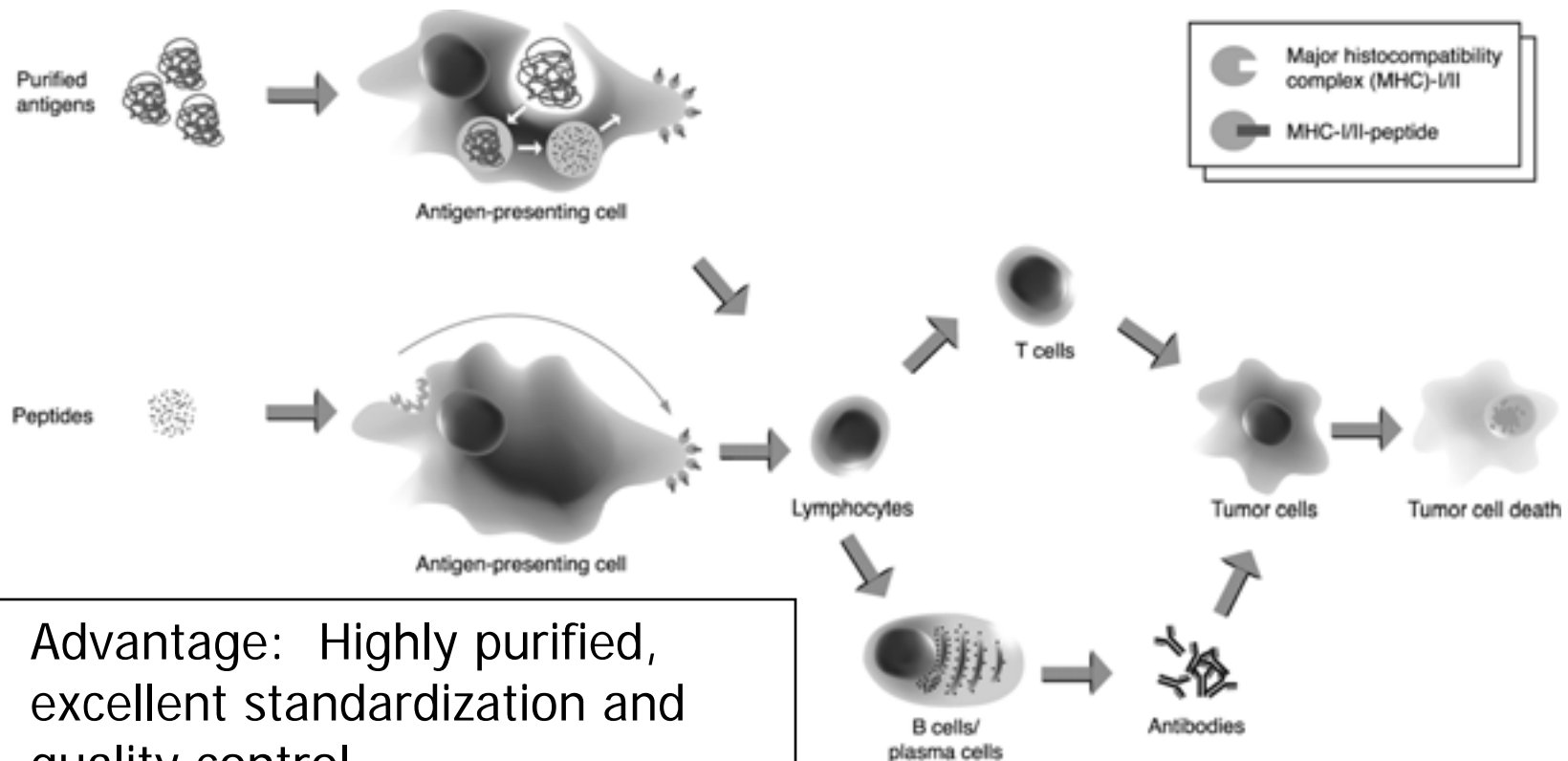
Active Immunotherapy

Breast Cancer Antigen
Vaccines

The Antitumor Immune Response



Antigen-Specific Vaccines in Breast Cancer

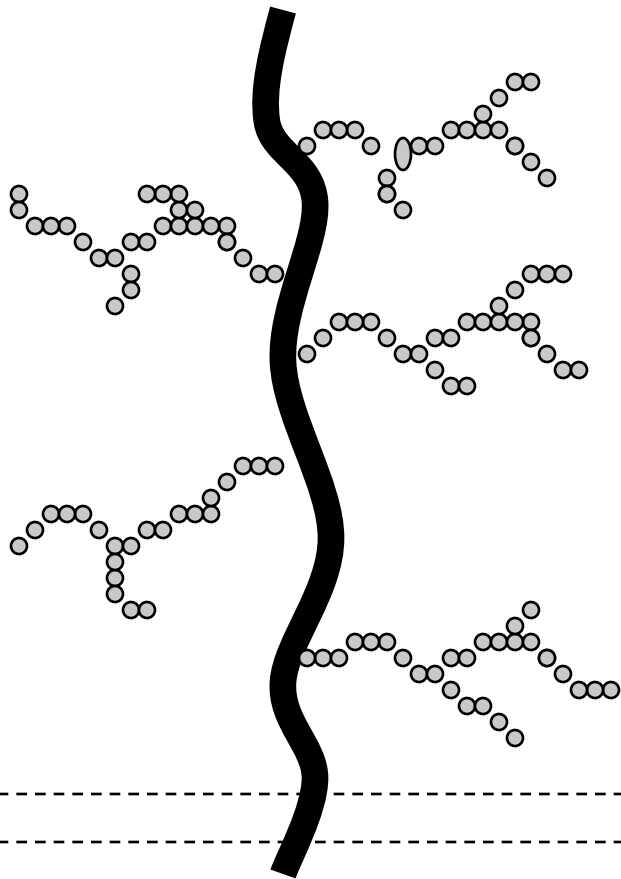


- Advantage: Highly purified, excellent standardization and quality control.
- Disadvantage: Less immunogenic, vulnerable to antigen modulation, HLA restricted

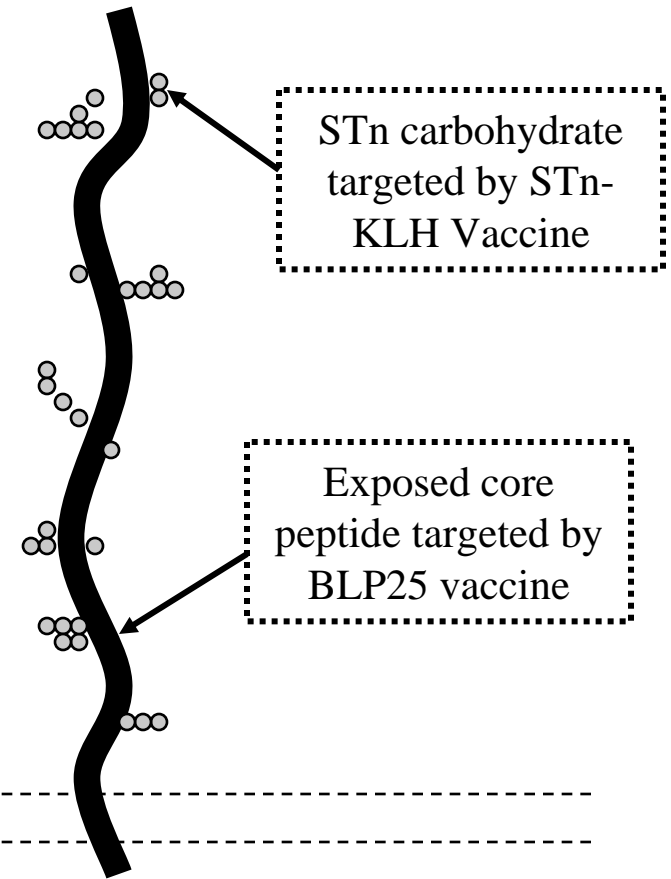
Tumor-Associated Antigen: MUC-1 Mucin

Highly Expressed in Aberrant Form on Most Cancers

Normal Mucin



MUC-1 Cancer Mucin



PLASMA
MEMBRANE

MUC-1 Vaccines

MUC-1 Peptide with Detox adjuvant in 16 pts with stage IV breast CA.

- Anti-MUC-1 Ab in 3/16 patients
- anti-MUC-1 CTL activity in 7/11 patients.

MUC-1 Peptide with BCG adjuvant in 108 pts with advanced breast, colon or pancreas CA.

- 37/108 patients had intense T-cell infiltration.

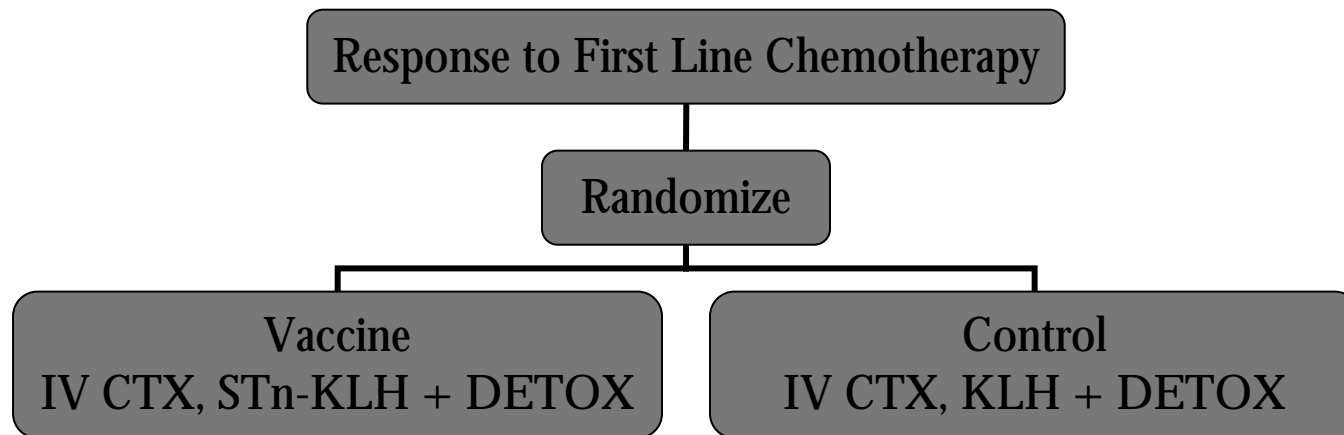
MUC-1-Mannan fusion protein vaccine in 25 pts with stage IV breast, colon or stomach CA.

- Anti-MUC-1 Ab in 13/25 patients.

STn-KLH (Theratope) Phase II Trials

- STn conjugated to Keyhole Limpet Hemocyanin (STn-KLH or **THERATOPE**)
- Minimal visible tumor response
- Improved Survival in High IgG Responders compared to Low IgG Responders
- Increased Antibody Levels With Chemotherapy (none vs. IV or PO)

Theratope Phase III Trial in Metastatic Breast Cancer

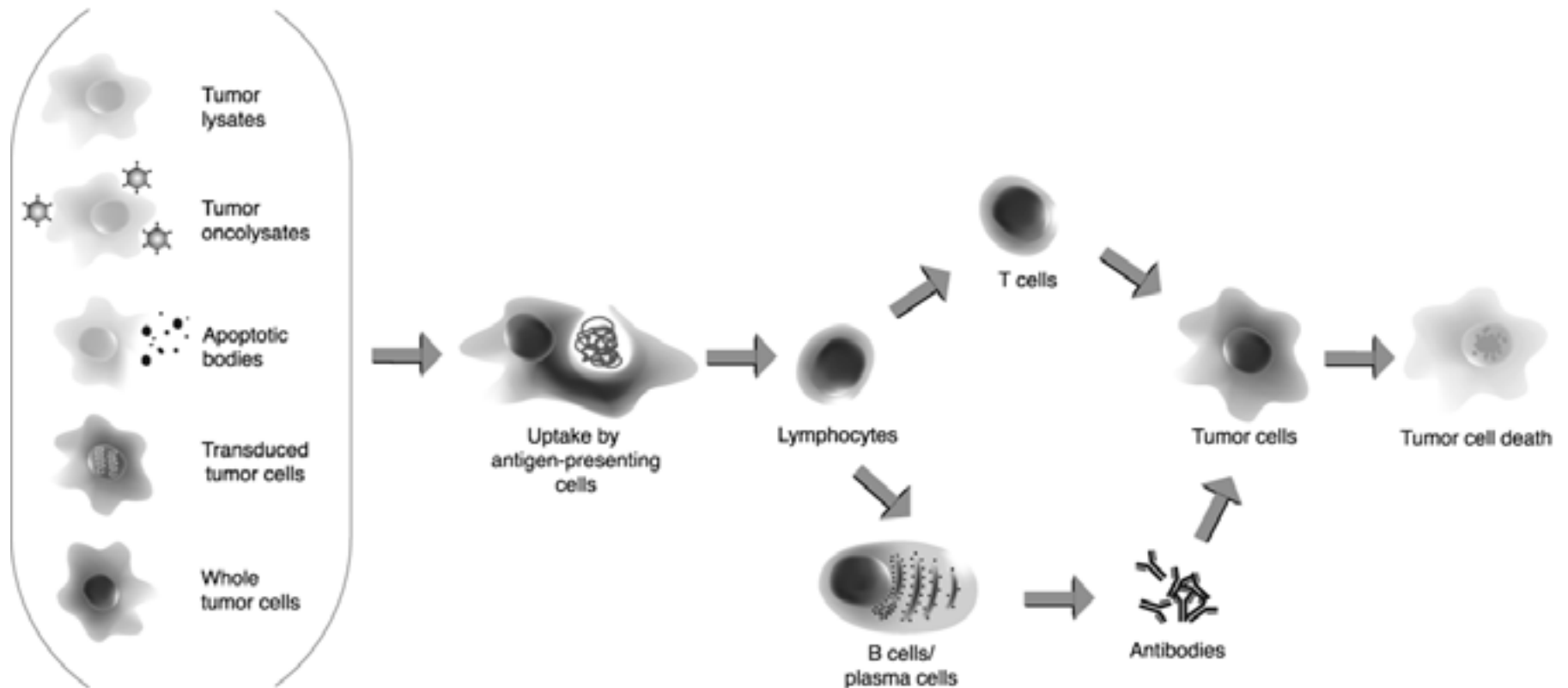


- 1,028 patients, powered to detect a 30% survival improvement.
- No difference in overall survival or time to progression
- Trend towards increased time to progression in patients on hormonal therapy.

Active Immunotherapy

Breast Cancer Cellular
Vaccines

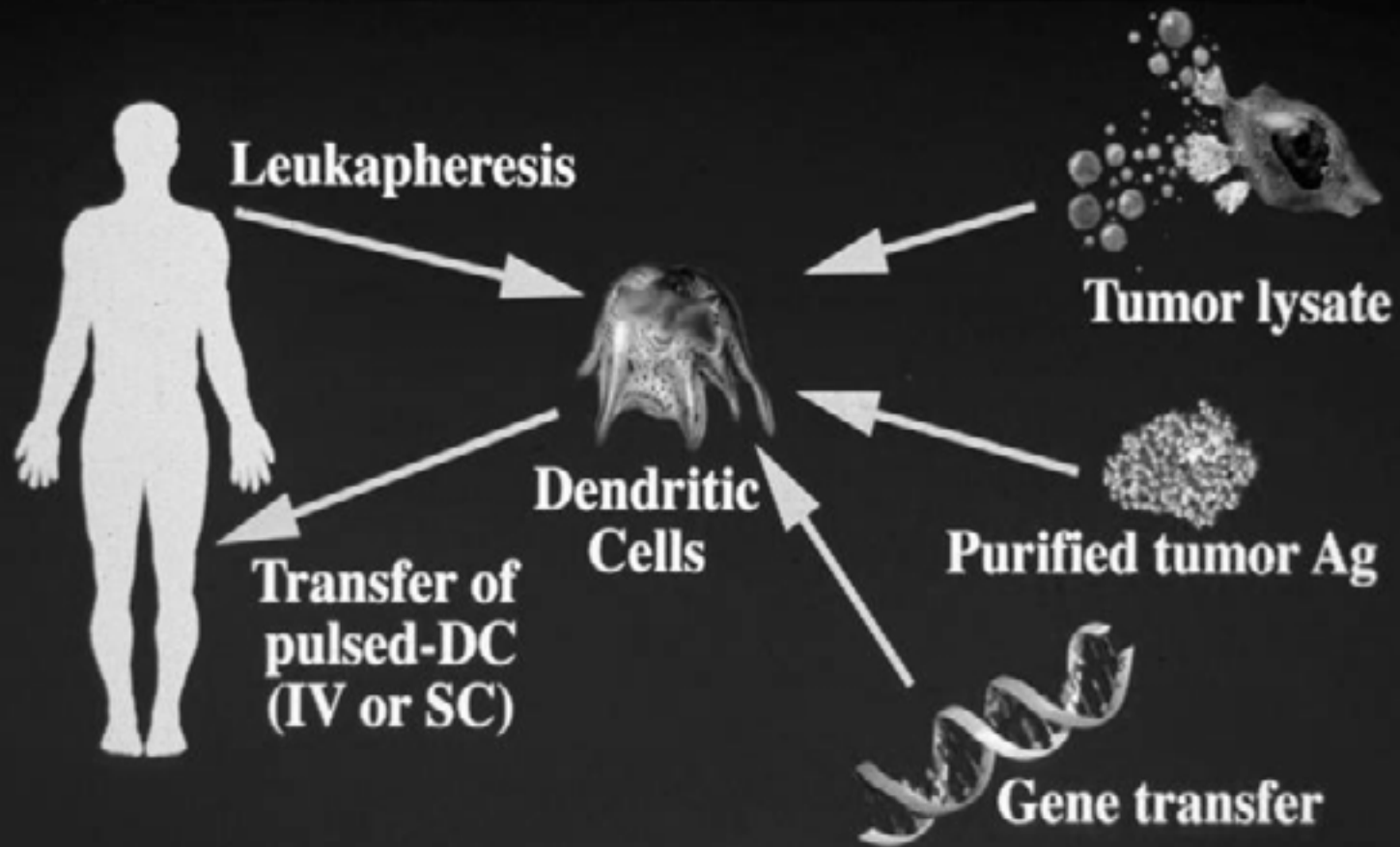
Sources of Antigens for Vaccines Stimulating Cell-Mediated Antitumor Immune Responses



Cellular Vaccines in Breast Cancer

- Advantages
 - More Immunogenic
- Disadvantages
 - Autologous Tumor Vaccines Difficult To Create
 - Accessing Adequate Tumor & Growing Cell Lines
 - Labor Intensive and Expensive
 - Allogeneic Tumor Vaccines Not Very Effective
 - Less common expression of antigens than melanoma
 - Poor results seen in phase II studies.

DENDRITIC CELL-BASED VACCINE



Obstacles to Successful Breast Cancer Immunotherapy

There are not enough shared antigens for a single bullet approach.

<u>Antigen</u>	<u>Expression</u>	<u>Type</u>
p53	17%	Mutational
CEA	50%	Differentiation
NY-BR-1	80%	Differentiation
HER-2/neu	30%	Amplified/Overexpressed
MUC-1	80%	Differentiation/Mutational
NY-BR-62	60%	Amplified/Overexpressed
NY-BR-85	90%	Amplified/Overexpressed
D52	60%	Amplified/Overexpressed
Mammoglobin	23%	Amplified/Overexpressed
NY-ESO-1	24%	Cancer-Testis Antigen
MAGE-3	14%	Cancer-Testis Antigen
SCP-1	30%	Cancer-Testis Antigen
SSX-1	12%	Cancer-Testis Antigen
SSX-4	14%	Cancer-Testis Antigen
CT-7	30%	Cancer-Testis Antigen

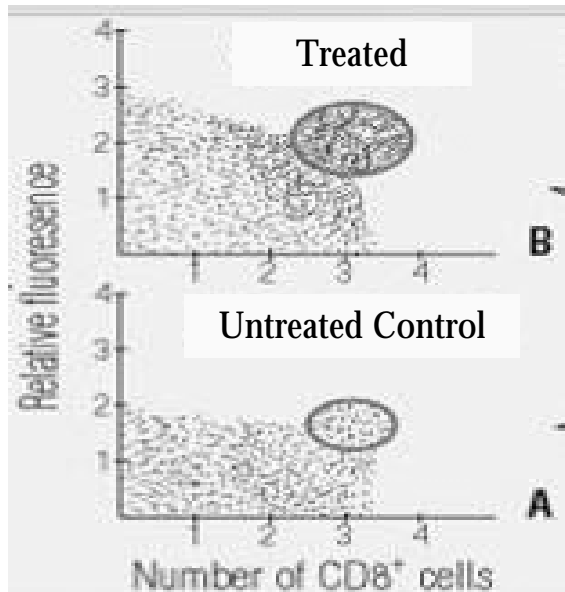
Approaches requiring significant tumor will not be clinically feasible.

- By 2010, 50% of breast cancers will be 1cm or less in size.
- After sectioning for margins, minimal tumor left.
- Extremely difficult to establish cell lines (10%) in vitro.
 - Needed for BOTH tumor vaccines (re-administration) and assays.
- Recurrence: Brain, bone, lung, liver.

Quantitating Immunologic Responses

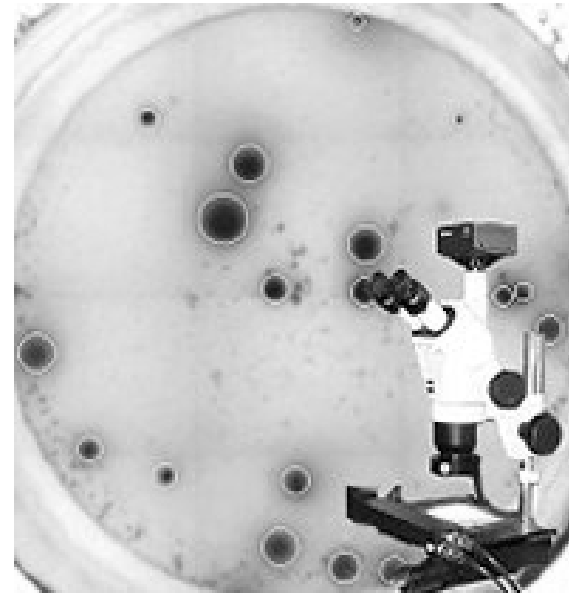
In Vivo Assays

- DTH
- Tumor Infiltration

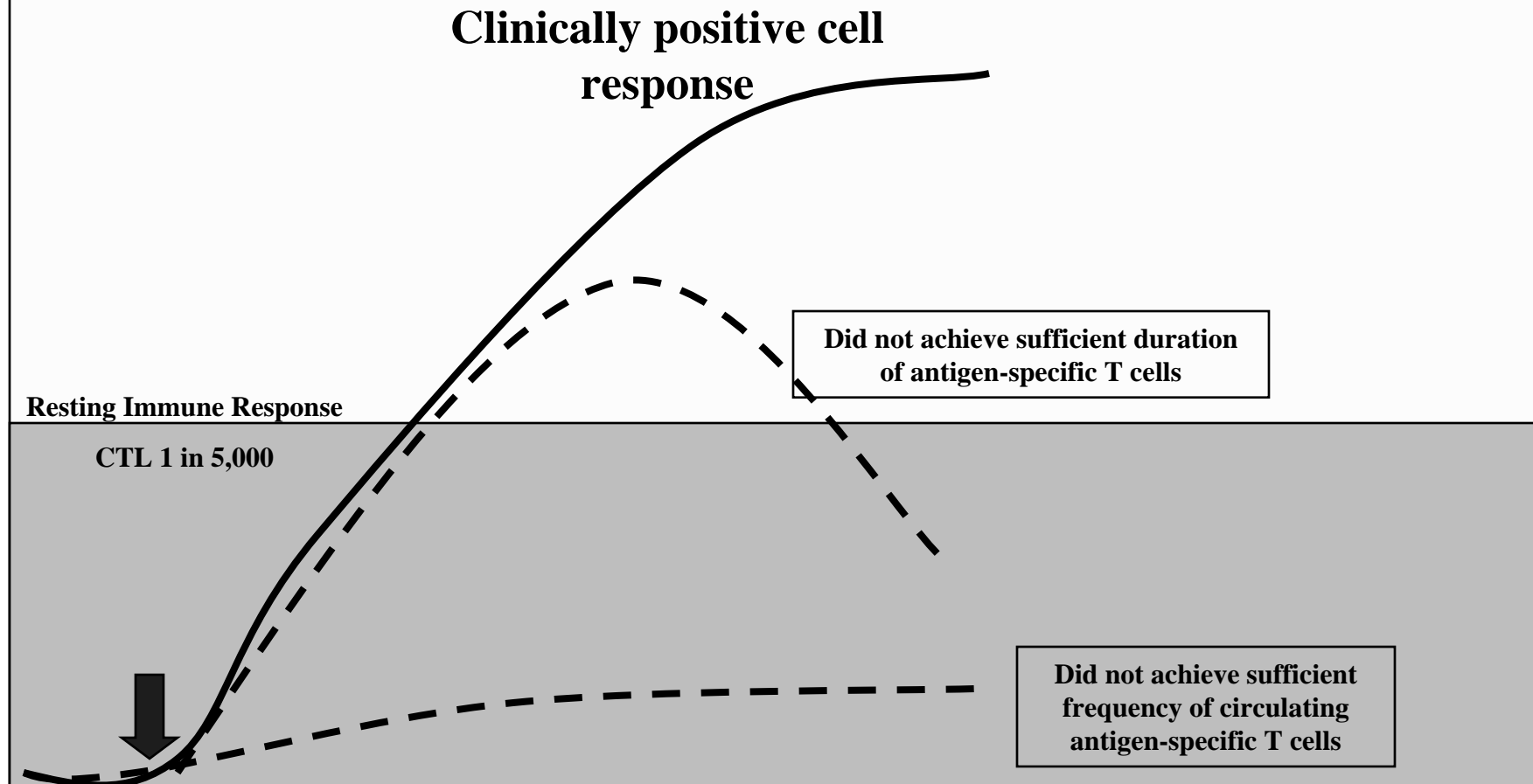


In Vitro Assays

- T-cell proliferation
- T-cell cytokine release
- ELISpot
- Peptide-MHC



Clinical Response vs. Immunologic Response



HLA Class I Antigen Loss

TABLE V

FREQUENCY OF HLA CLASS I ANTIGEN LOSS OR DOWN-REGULATION IN HUMAN CANCERS

Cancer Type	# Lesions	Total Loss (%)	Locus or Allelic Loss (%)	Combined (%)
Head and neck ^a	381	24	9	33
Breast ^b	524	43	11	54
Lung ^c	366	27	4	31
Renal ^d	264	16	16	32
Colon ^e	794	27	16	43
Cervical ^f	488	29	26	55
Prostate ^g	118	50	20	70
Melanoma ^h	569	16	35	51



Marincola et al. (2000) Adv in Immunol. 74:181-273

Tumor Antigen Heterogeneity of Disseminated Breast Cancer Cells in Minimal Residual Disease

TABLE II – HETEROGENEOUS EXPRESSION OF TUMOR-ASSOCIATED CELL-SURFACE ANTIGENS ON ISOLATED BREAST CANCER CELLS IN BONE MARROW

Patient	Number of target ⁺ /CK ⁺ cells per total number of CK ⁺ cells (%)				
	c-erbB-2	CO17-1A	MUC-1	Lewis ^Y	Antibody cocktail ¹
Stage M ₀					
002	182/516 (35.2)	155/471 (33.1)	128/340 (37.7)	74/248 (29.8)	204/481 (42.4)
280	39/88 (44.3) ²	33/48 (68.7)	26/53 (49.1) ²	20/42 (46.5) ²	51/65 (78.5)
287	38/58 (60.3) ²	19/35 (54.3)	37/77 (48.1) ²	0/5 ²	14/14 (100)
385	85/167 (50.8)	71/155 (45.8)	41/84 (48.8)	64/146 (43.8)	62/12 (50.8)
Stage M ₁					
001	47/134 (34.0)	56/199 (28.1)	36/138 (26.1)	12/82 (14.6) ²	55/161 (34.2)
295	440/965 (45.6)	370/1,348 (27.5)	481/975 (49.3)	533/988 (54.0)	441/799 (55.2)
313	—	25/49 (65.3)	0/16 ²	—	21/32 (65.6)
326	11/12 (71.7)	9/12 (75.0)	6/19 (31.6) ²	11/19 (57.9) ²	47/47 (100)
393	0/7 ²	1/3 (33.3)	0/1 ²	1/4 (25.0) ²	2/4 (50.0)
449	1/9 (11.1) ²	3/8 (37.5) ²	3/5 (60.0) ²	6/12 (50.0) ²	7/7 (100)
454	9/20 (45.0)	10/21 (47.6)	13/33 (39.4) ²	9/26 (34.6) ²	12/14 (85.7)
520	1,670/3,874 (43.1)	2,335/3,576 (65.3)	2,650/4,804 (55.2) ²	2,685/4,570 (58.8)	4,073/4,748 (85.8)
536	0/9 ²	2/30 (6.7) ²	10/16 (62.5)	—	7/11 (63.6)
716	756/3,260 (23.2) ²	154/2,975 (5.2) ²	2,345/3,567 (65.7) ²	39/2,870 (1.4) ²	2,980/3,320 (89.8)

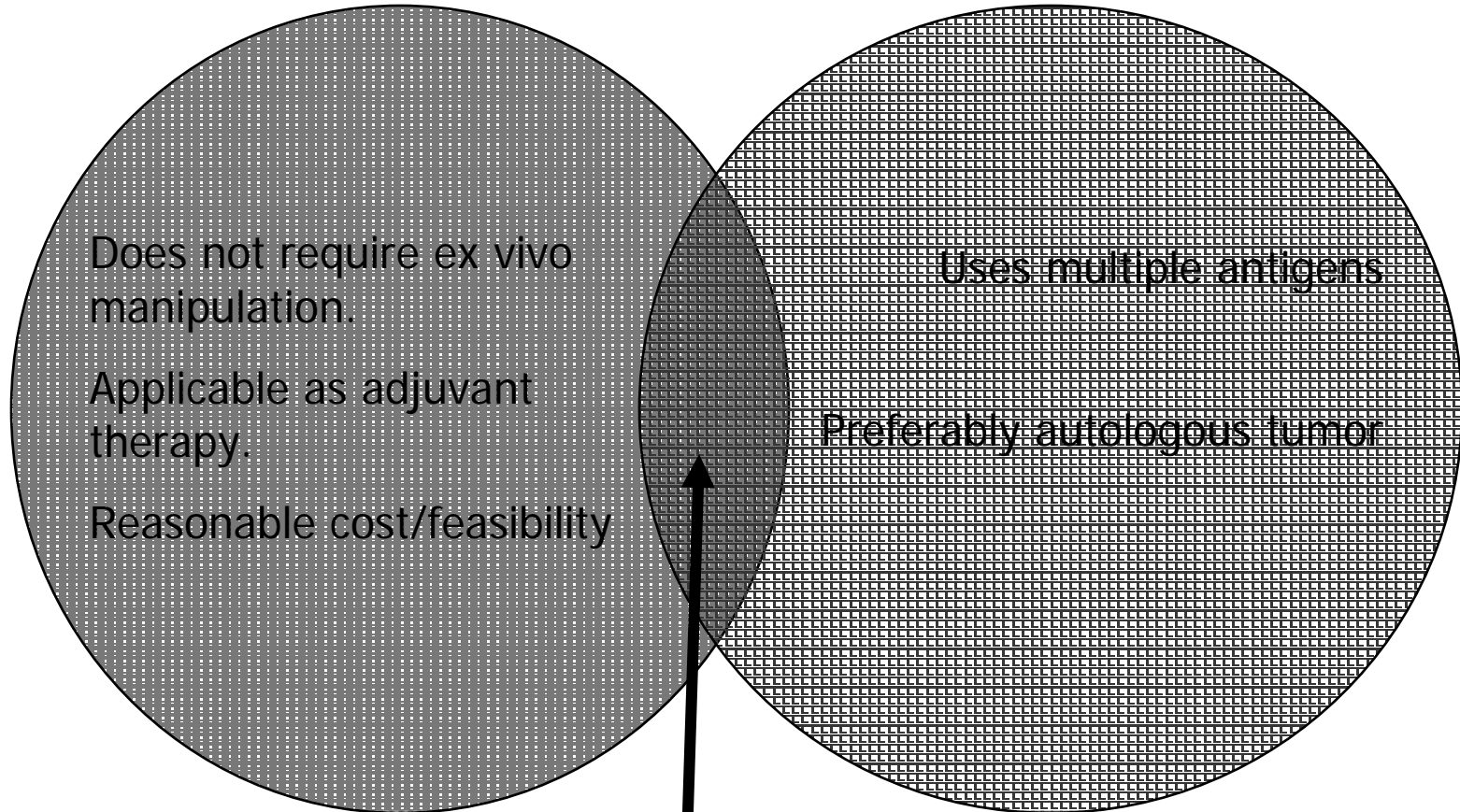
¹Contained MAbs to c-erbB-2, CO17-1A, MUC-1 and Lewis^Y. ²Number of CK⁺ cells co-labeled by antibody cocktail was 50% or more higher than that labeled by any single MAb.

Tumor Escape Mechanisms

- Loss of antigen expression
- Loss of MHC processing/expression
- Anergy/Tolerance
- Regulatory T lymphocytes (T_{reg} cells)
- Tumor Immunosuppression
 - TGF- β
 - IL-10, IL-4 (Th2 response)
 - Lymphocyte apoptosis (Fas-Fas Ligand)

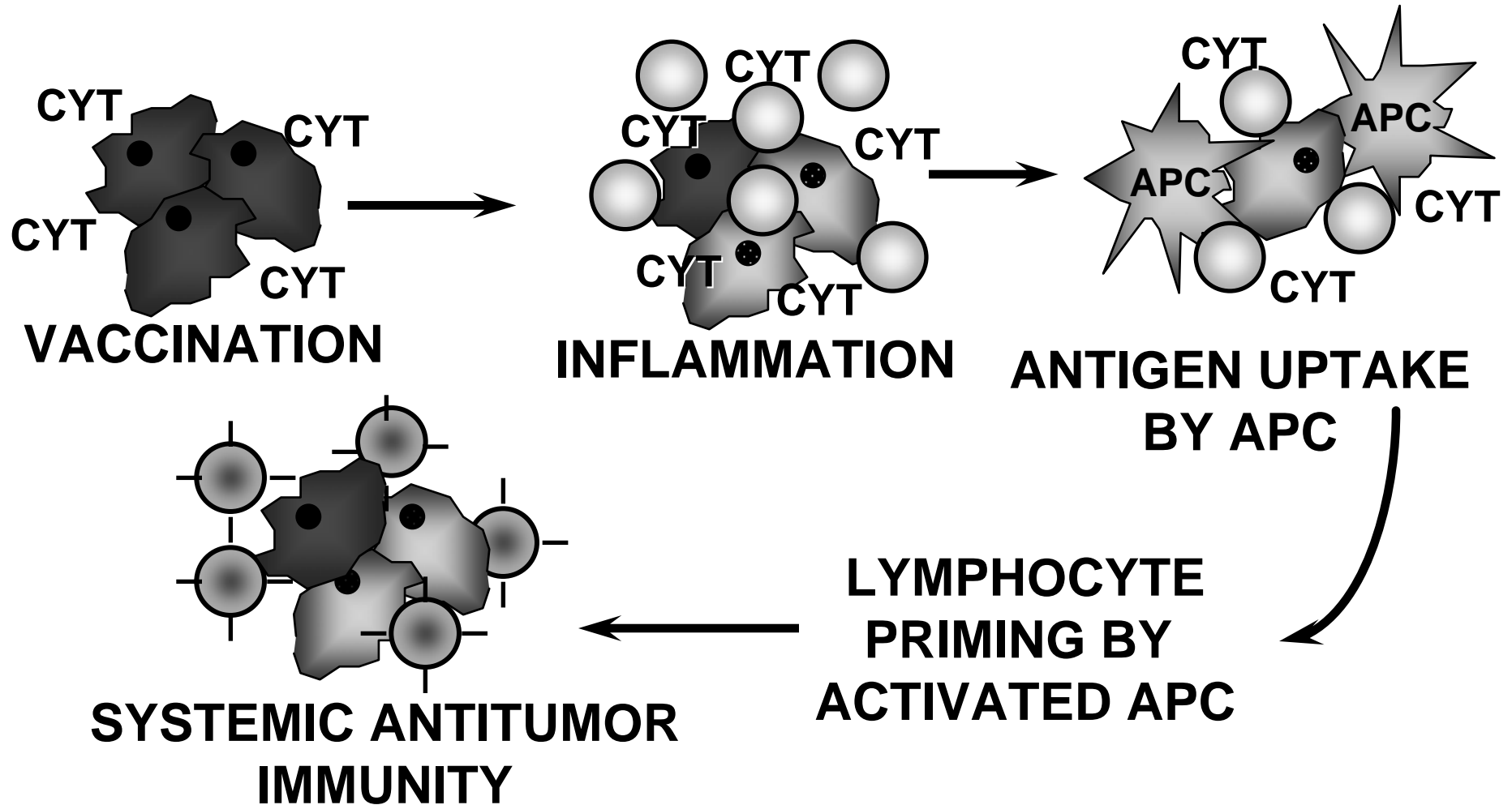
Clinically Feasible

Sufficiently Immunogenic



Neoadjuvant Immunotherapy

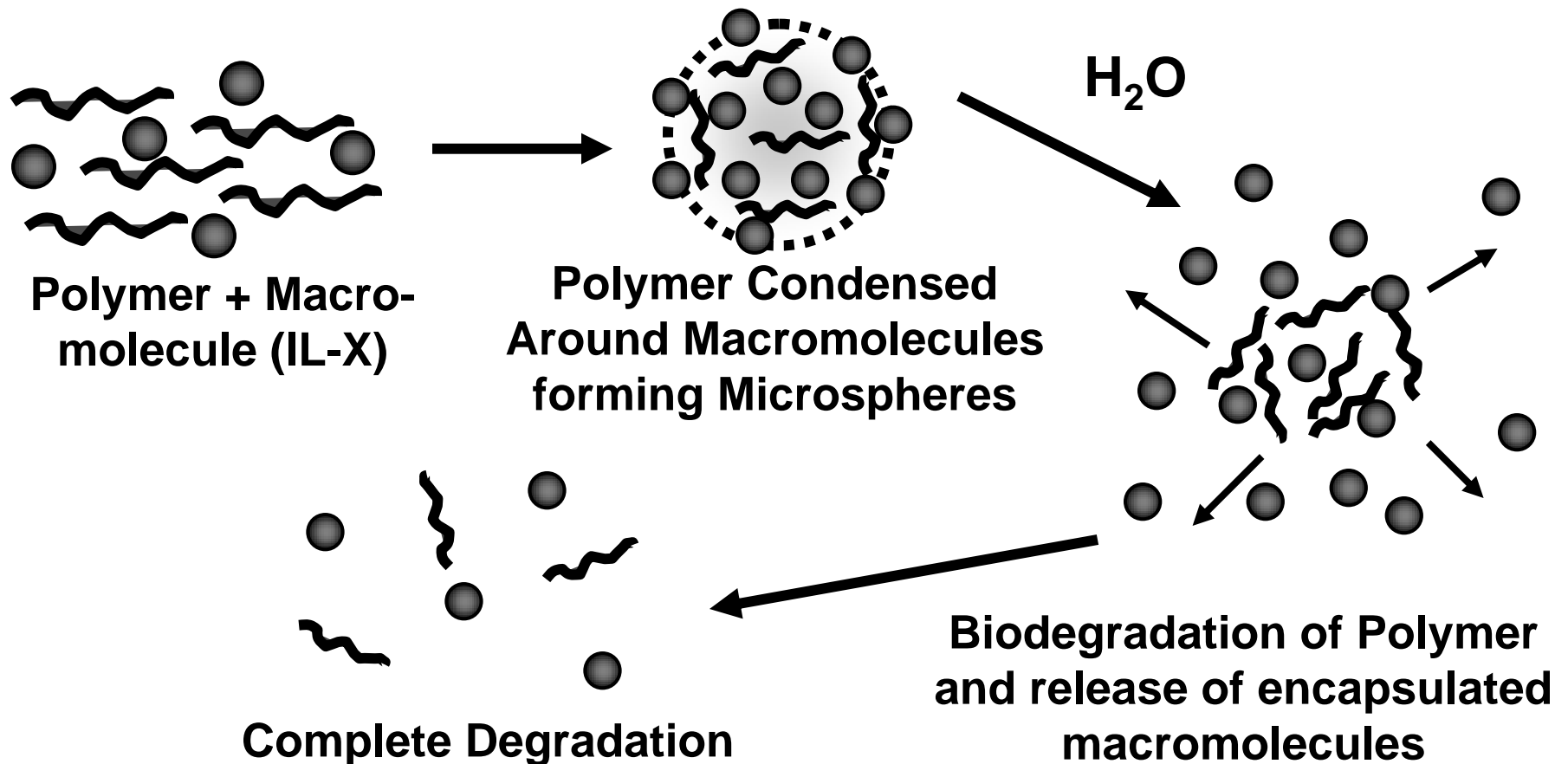
NEOADJUVANT IMMUNOTHERAPY or *IN SITU* TUMOR VACCINATION:



Intratumoral Delivery of Cytokines

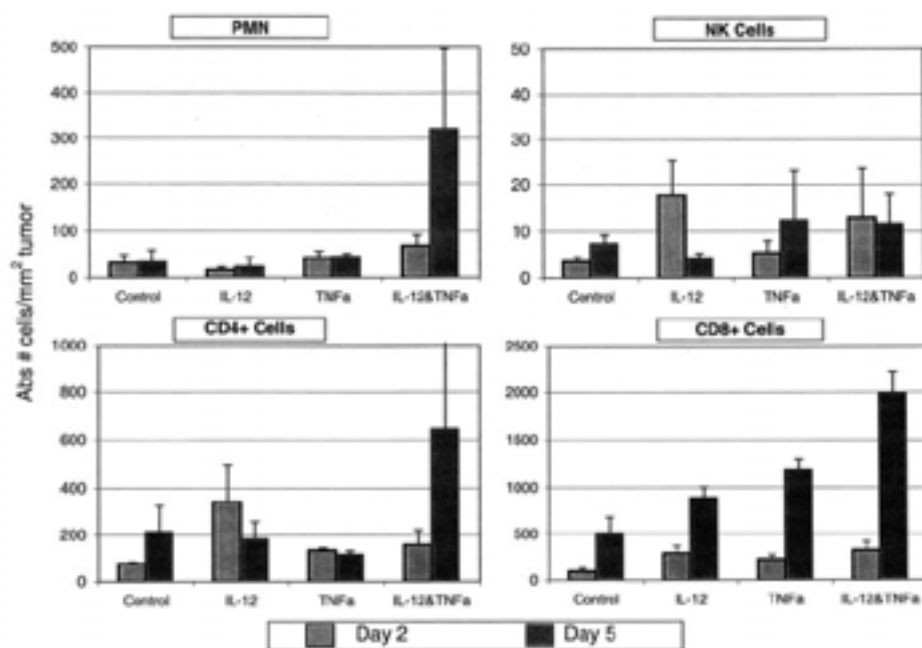
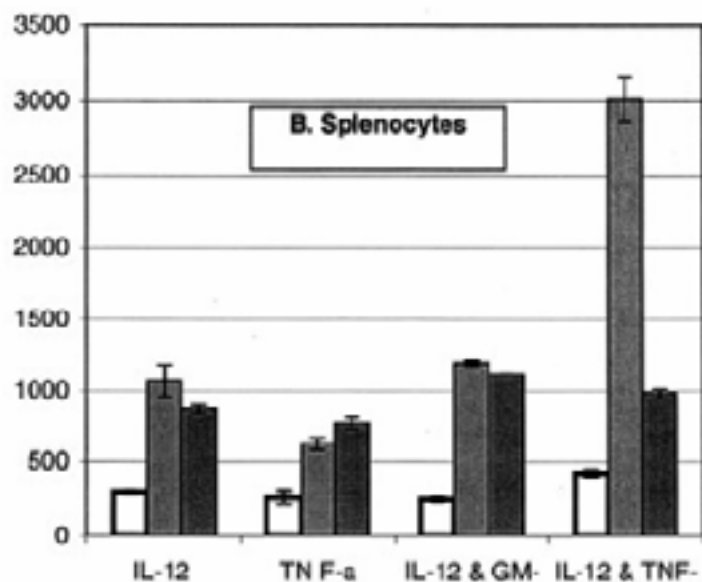
- Injection of Free Cytokine
- Genetically Modified Cells
 - Fibroblasts
 - Dendritic Cells
- Adenoviral Delivery of Cytokine Genes
- Biodegradable Polymers

Biodegradable Poly-Lactic Acid Microspheres

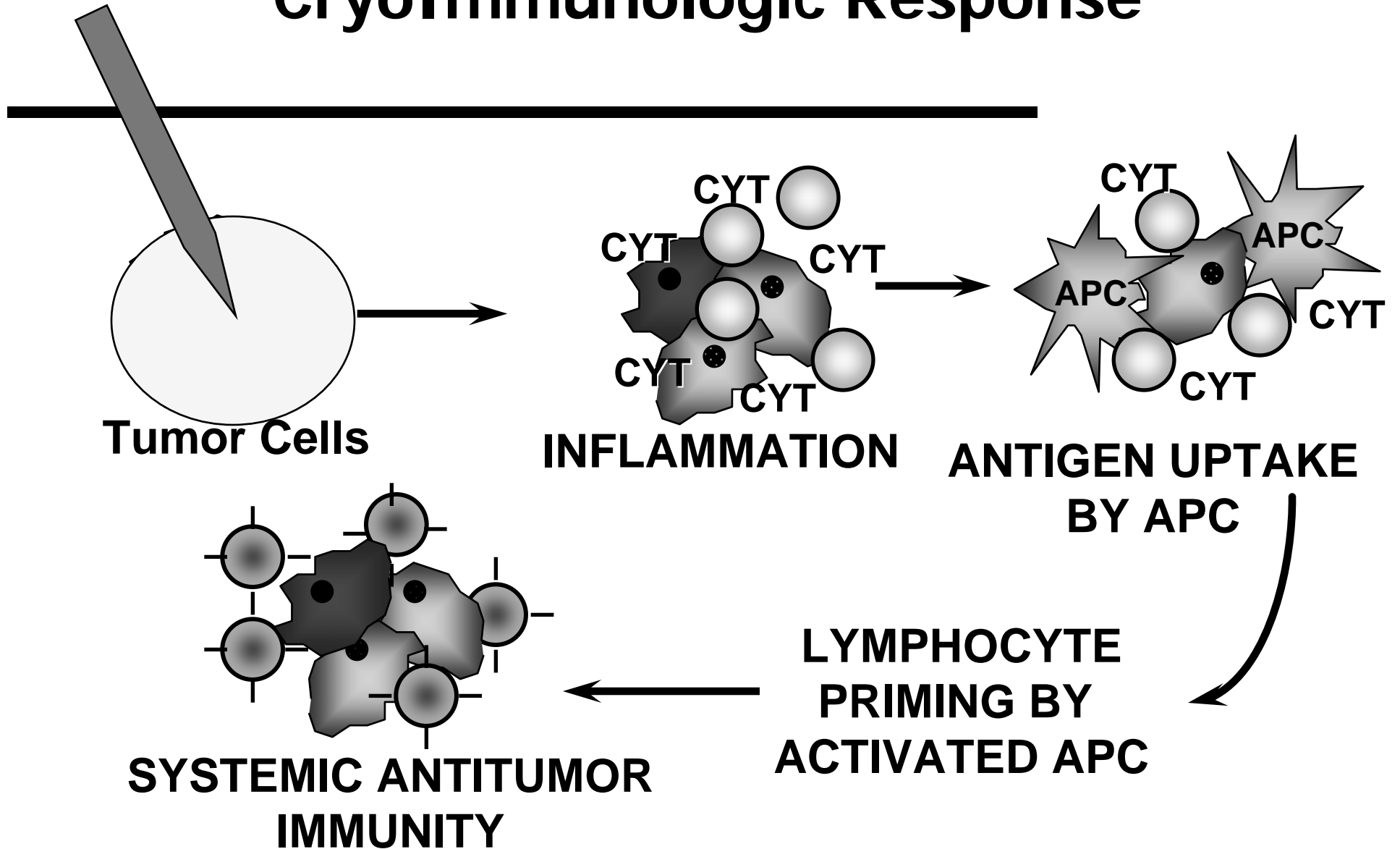


Intratumoral IL-12 and TNF- α -Loaded Microspheres Lead To Regression of Breast Cancer and Systemic Antitumor Immunity

Michael S. Sabel, MD, Joseph Skitzki, MD, Lloyd Stoolman, MD, Nejat K. Egilmez, PhD, Edith Mathiowitz, PhD, Nicola Bailey, PhD, Wen-Jian Chang, MD, and Alfred E. Chang, MD



Cryoimmunologic Response





Cryoablation of Early-Stage Breast Cancer: Work-in-Progress Report of a Multi-Institutional Trial

Michael S. Sabel, MD, Cary S. Kaufman, MD, Pat Whitworth, MD, Helena Chang, MD, PhD,
Lewis H. Stocks, MD, PhD, Rache Simmons, MD, and Michael Schultz, MD

TABLE 3. *Correlation of tumor size and histology with successful cryoablation*

Treatment group	No. Patients	No residual invasive or DCIS ^a
All patients	27	21 (78%)
Tumors <1.0 cm (all histology)	11	11 (100%)
Tumors >1.0 cm (all histology)	16	10 (63%)
Any size lobular or colloid	5	2 (40%)
Any size ductal with EIC	5	3 (60%)
Any size ductal or medullary, no EIC	17	15 (88%)
Tumors <1.5 cm; ductal or medullary, no EIC	10	10 (100%)
Tumors >1.5 cm; ductal or medullary, no EIC	7	5 (71%)

DCIS, ductal carcinoma-in-situ; EIC, extensive intraductal component.

^a Excludes foci of DCIS found in healthy tissue surrounding the treatment zone.

Anecdotal Human Evidence of Anti-Tumor Immunity Following Cryotherapy

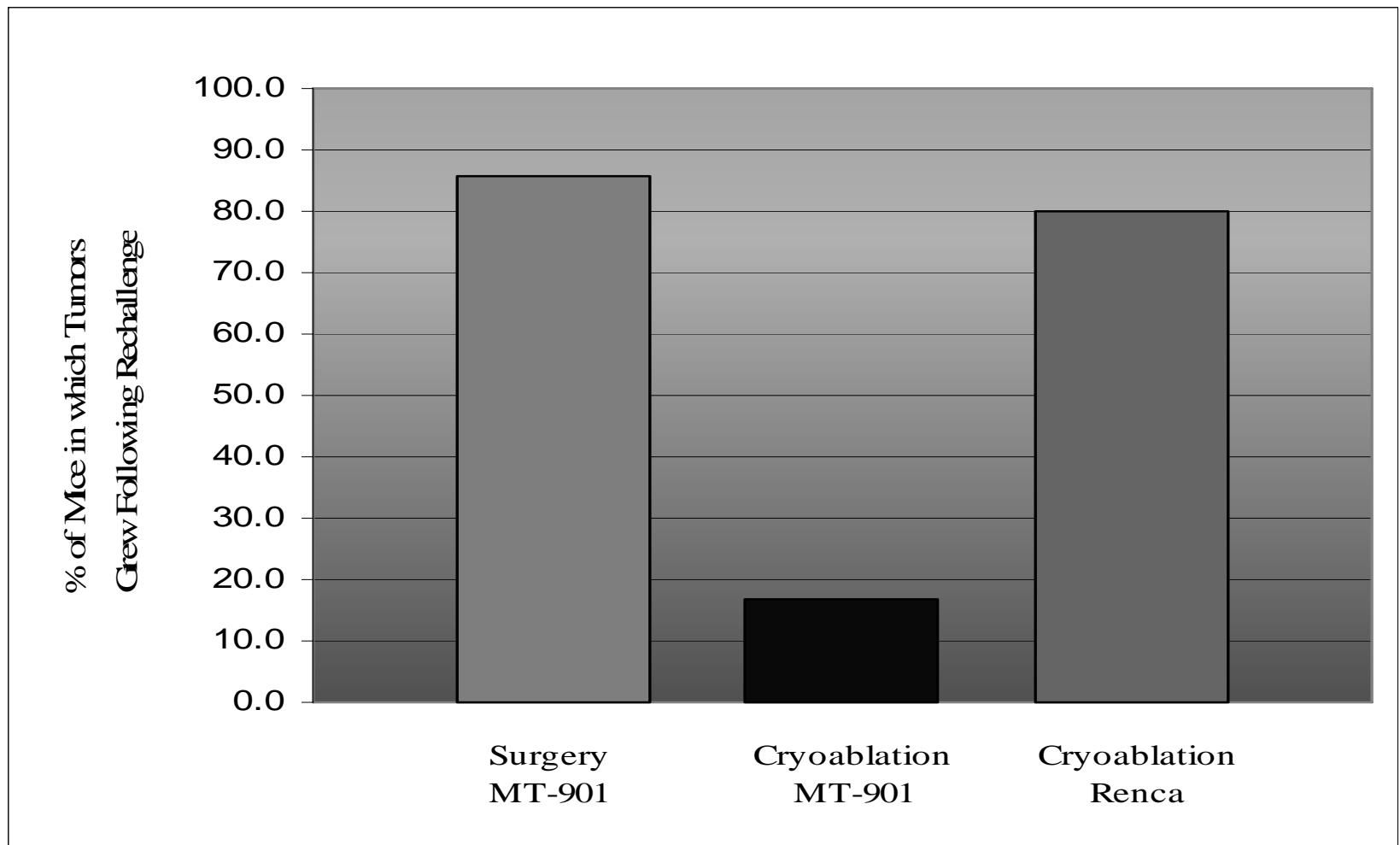
- Regression of lung and SC nodes in 3 patients after cryoablation of prostate CA.
 - Soanes WA, et al. J Urol 1970;104:154-159.
- 2 of 8 patients with advanced breast cancer with regression of lymph node and lung metastatic lesions after cryoablation.
 - Suzuki Y. Skin Cancer 1995;10:19-26.
- 1 of 7 patients with locally advanced breast cancer with regression of regional nodes after cryoablation.
 - LePivart P. In Ablin RJ. Handbook of cryosurgery. New York, NY: Marcel Dekker Inc; 1980;15-68.

Report

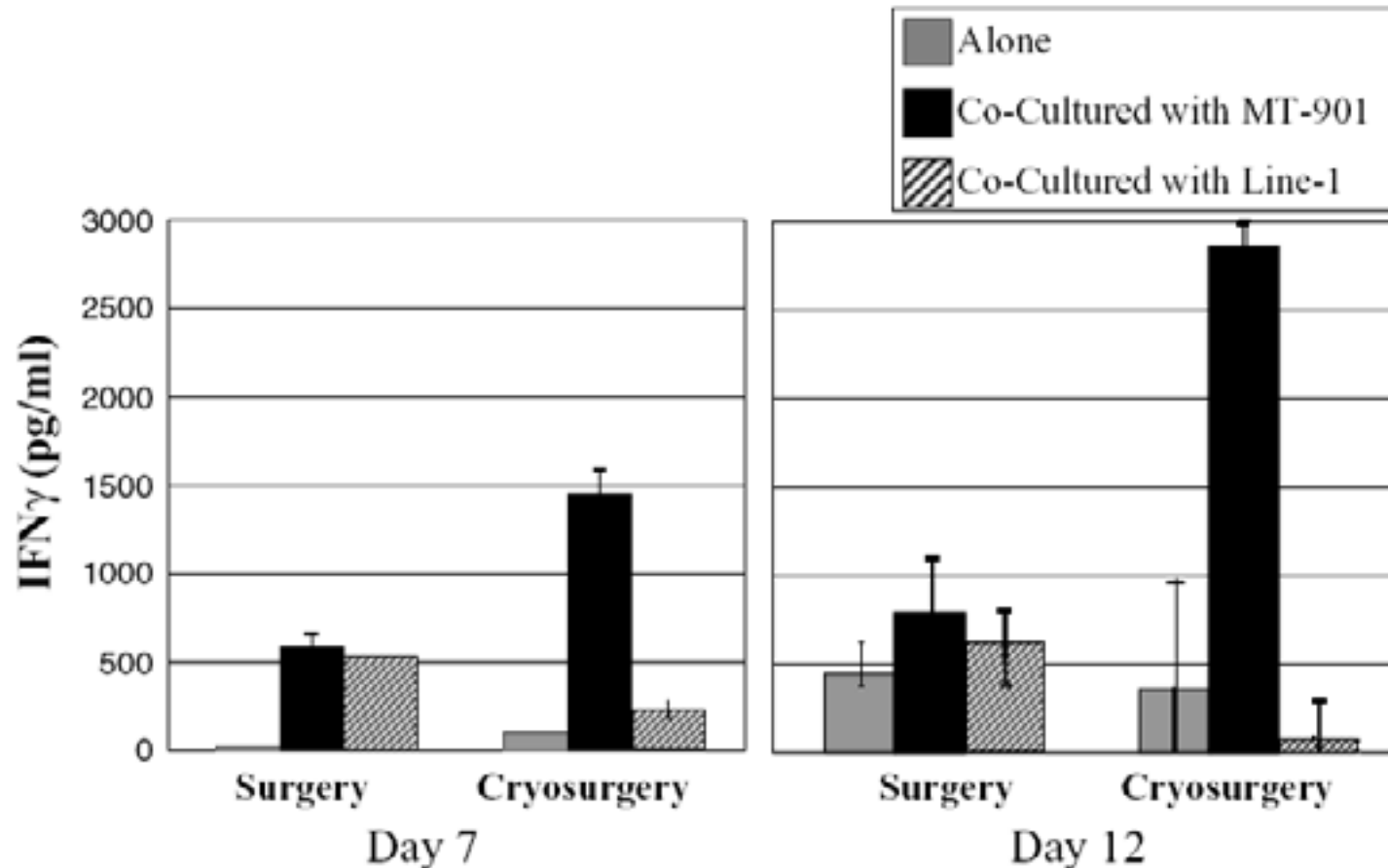
Immunologic response to cryoablation of breast cancer

Michael S. Sabel, Matthew A. Nehs, Gang Su, Kathleen P. Lowler, James L.M. Ferrara, and Alfred E. Chang

University of Michigan, 3304 Cancer Center, East Medical Center, Ann Arbor, MI, USA

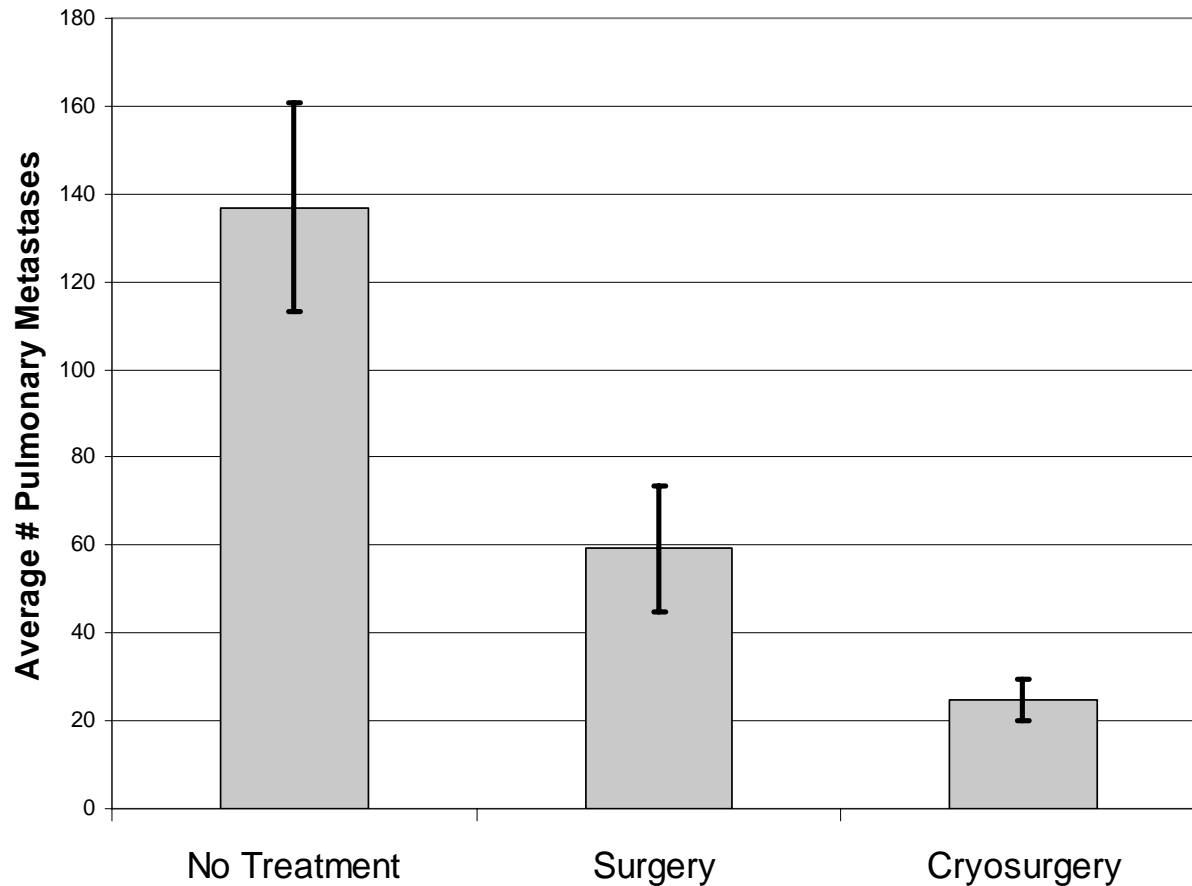


Cryoablation Results in Increased Tumor-Specific T-cells in the TDLN



Sabel et al. Immunologic response to cryoablation of breast cancer. Breast Cancer Research and Treatment 2005;90:97-104

Pulmonary Mets After Adoptive Transfer of TDLN after Cryosurgery vs. Surgery



Sabel MS, et al. Adoptive immunotherapy of breast cancer with lymph node cells primed by cryoablation of the primary tumor. *Cryobiology*. In Press.

Breast Immunotherapy

Conclusions

- Unique Challenges
 - Difficult to manipulate in lab.
 - Few common antigens.
 - Difficult to harvest tumor for vaccine production.
 - Small primary tumors, access of metastatic lesions
- Novel Approaches to Stimulating The Immune System Necessary
- Interactions of Immunotherapy with Radiation or Chemotherapy

An aerial, black and white photograph of a city, likely a university campus, with various buildings and green spaces. The text is overlaid on the image.

The End

*The Best Way To Predict The Future...
Is To Invent It*