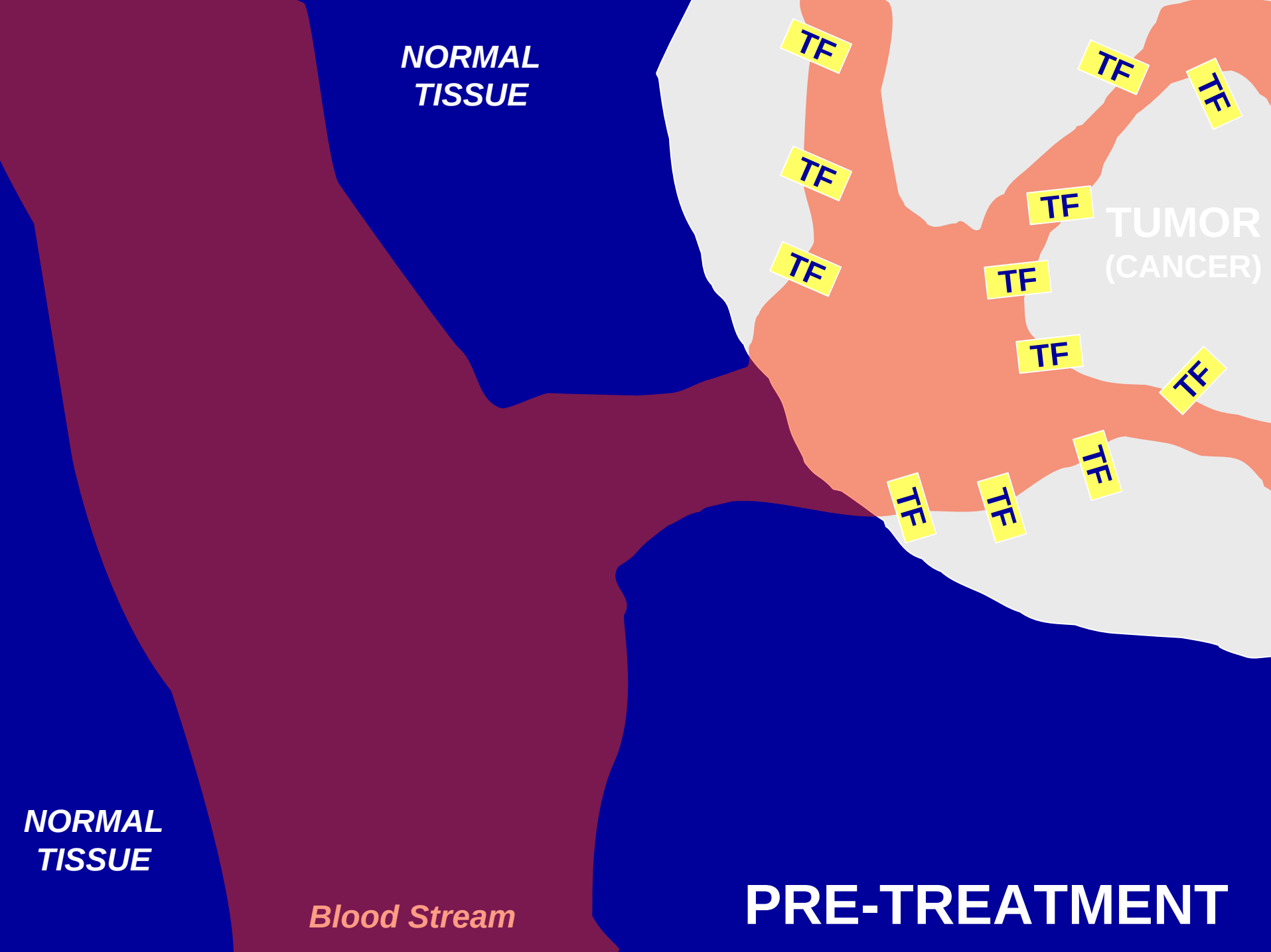
Background

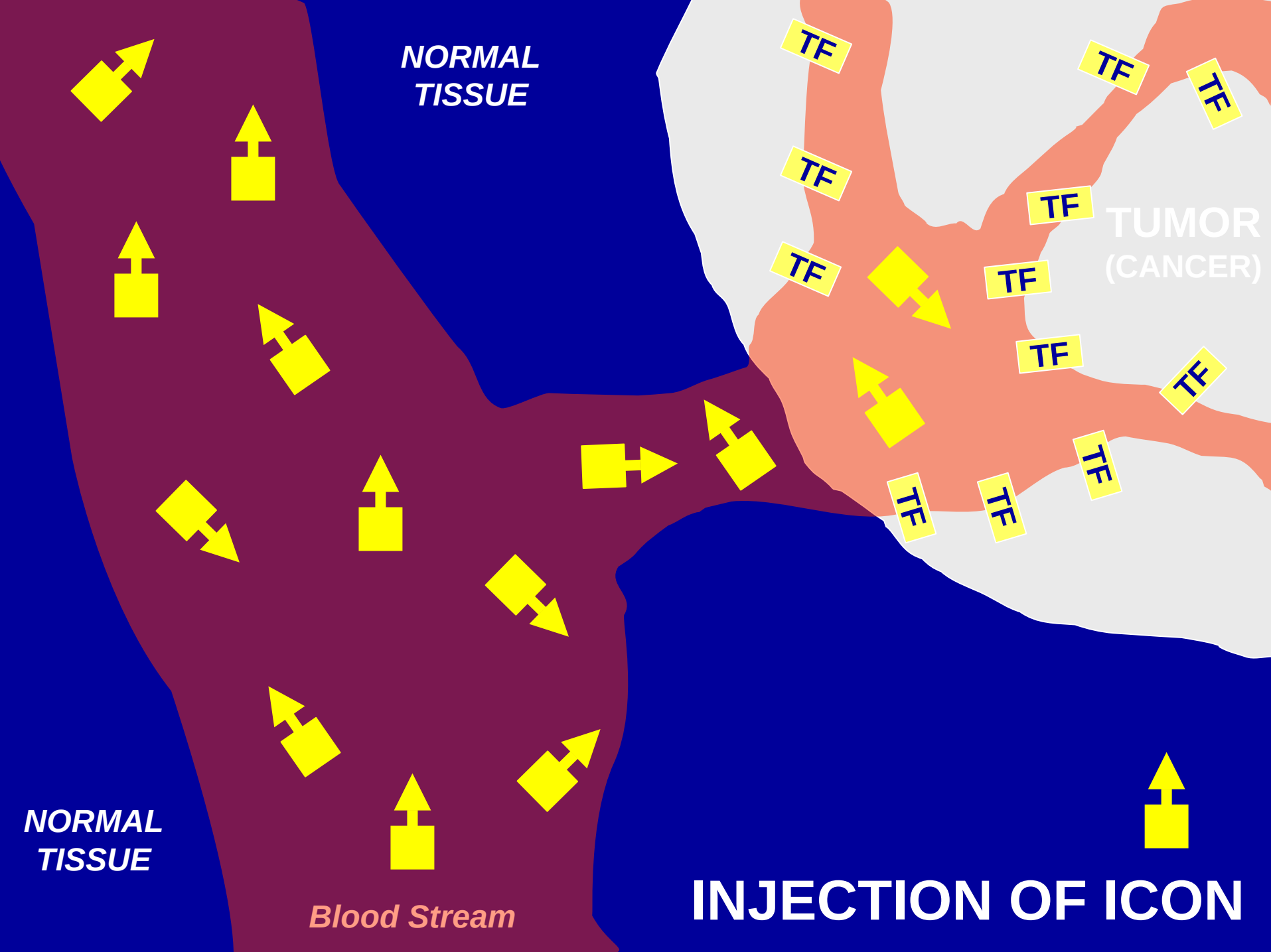
Garen had used a human tumor xenograft model to show that an immunoconjugate molecule composed of a mutated form of mouse factor VII linked to IgGFc induced regressions of tumor nodules and tumor vasculature (PNAS 96:8161, 1999;PNAS 97:9221, 2000; PNAS 98:12180, 2001).

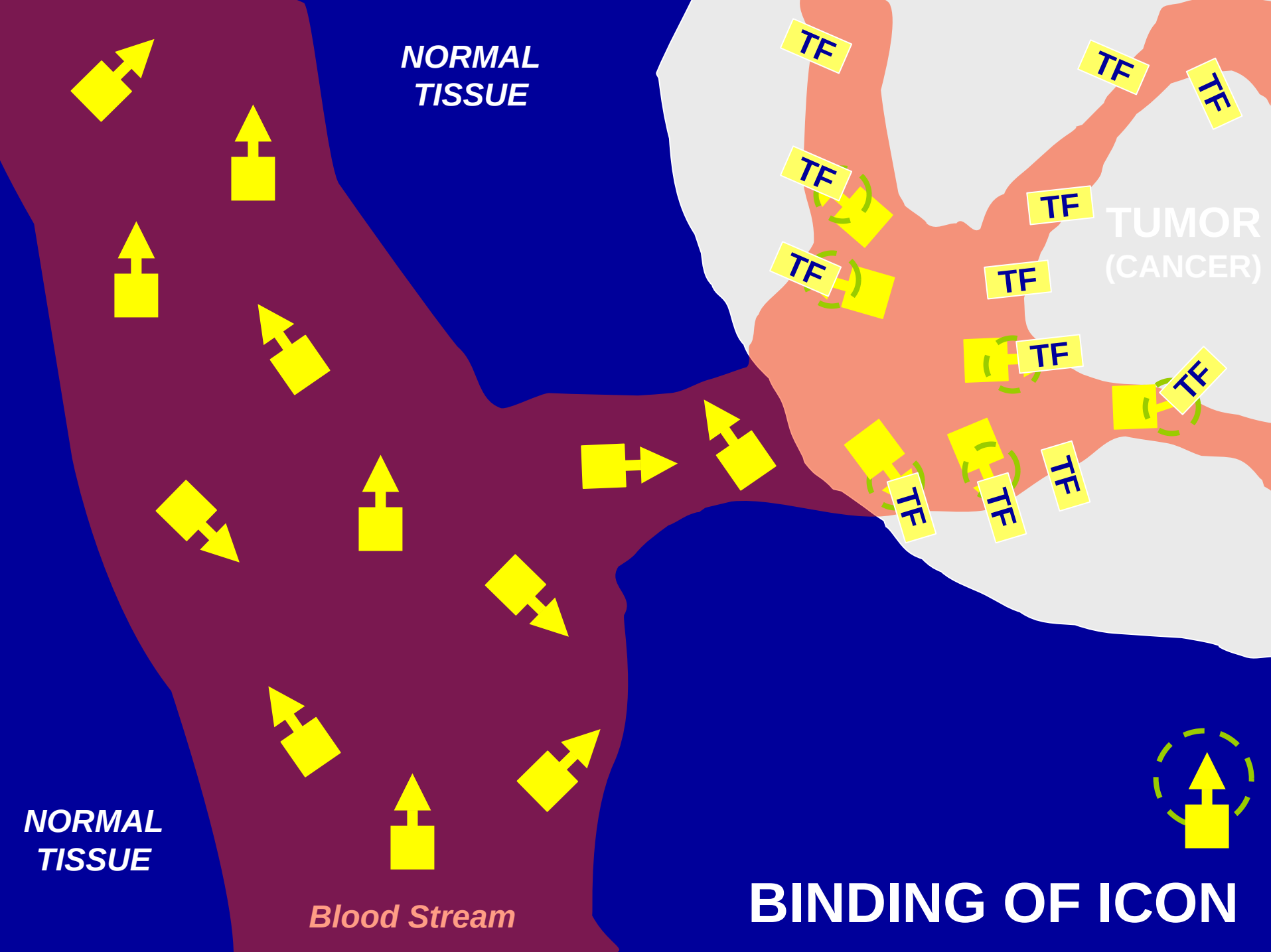
This immunoconjugate molecule is called the “Icon”.

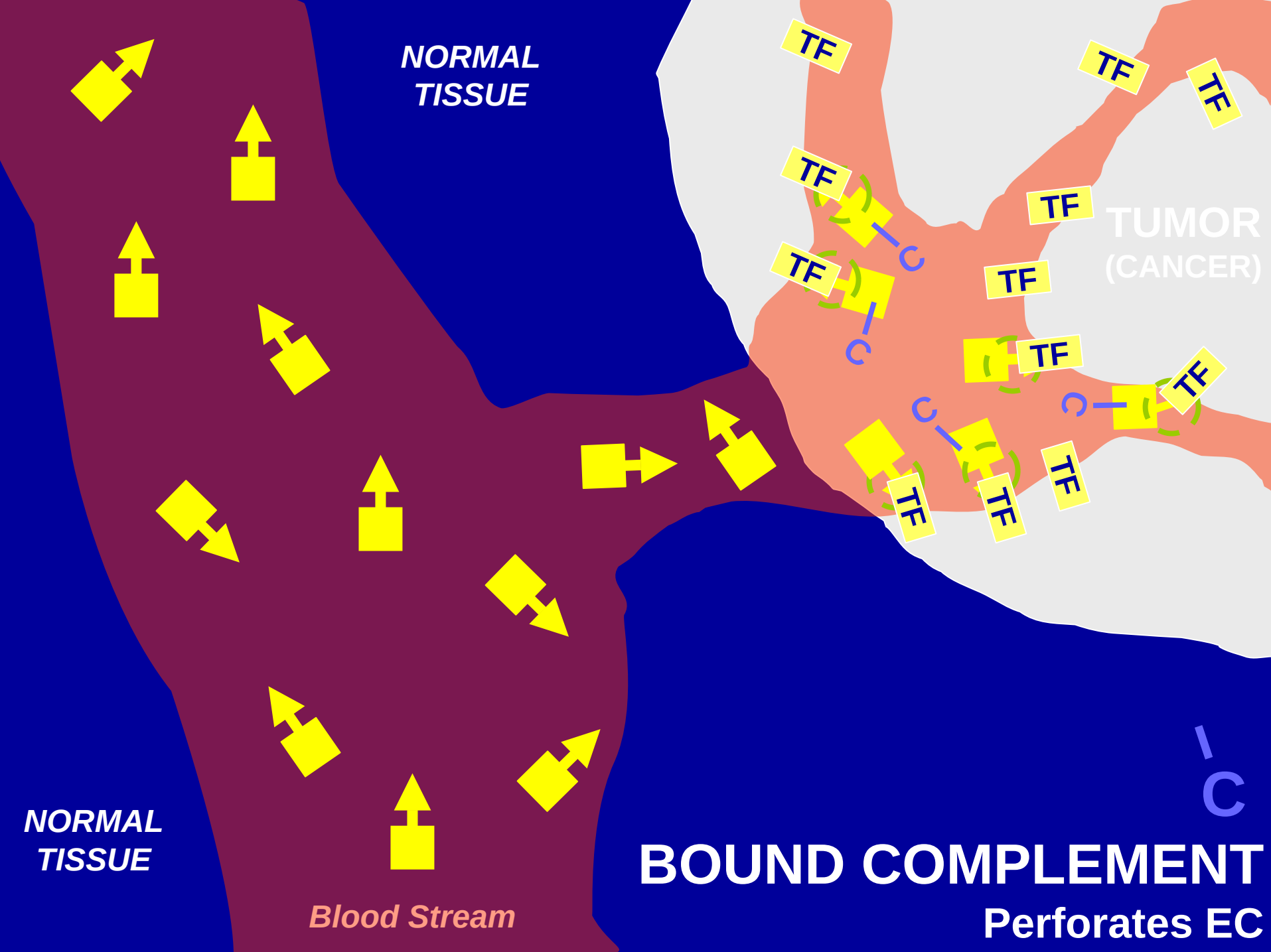
Background Continued

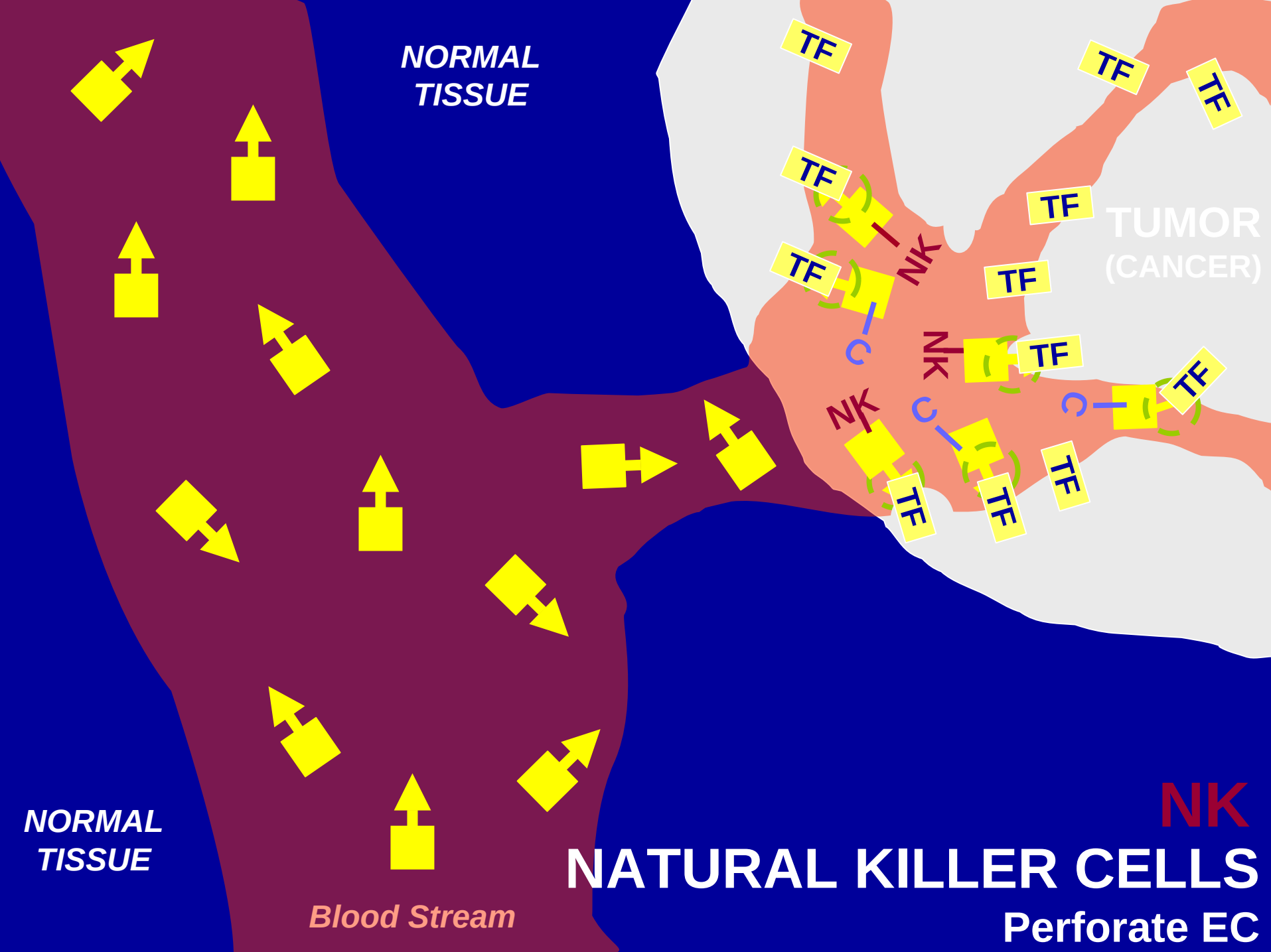
- Although Tissue Factor is found in the walls of most vessels, it is not exposed on the luminal surface of endothelial cells of normal vasculature. The binding selectivity of the Icon for cancer tissue and tumor vasculature is based on the presence of Tissue Factor on the luminal surface of tumor vasculature and tumor cells. Binding of the Icon induces fixation of complement and binding of NK cells to tumor vessels.

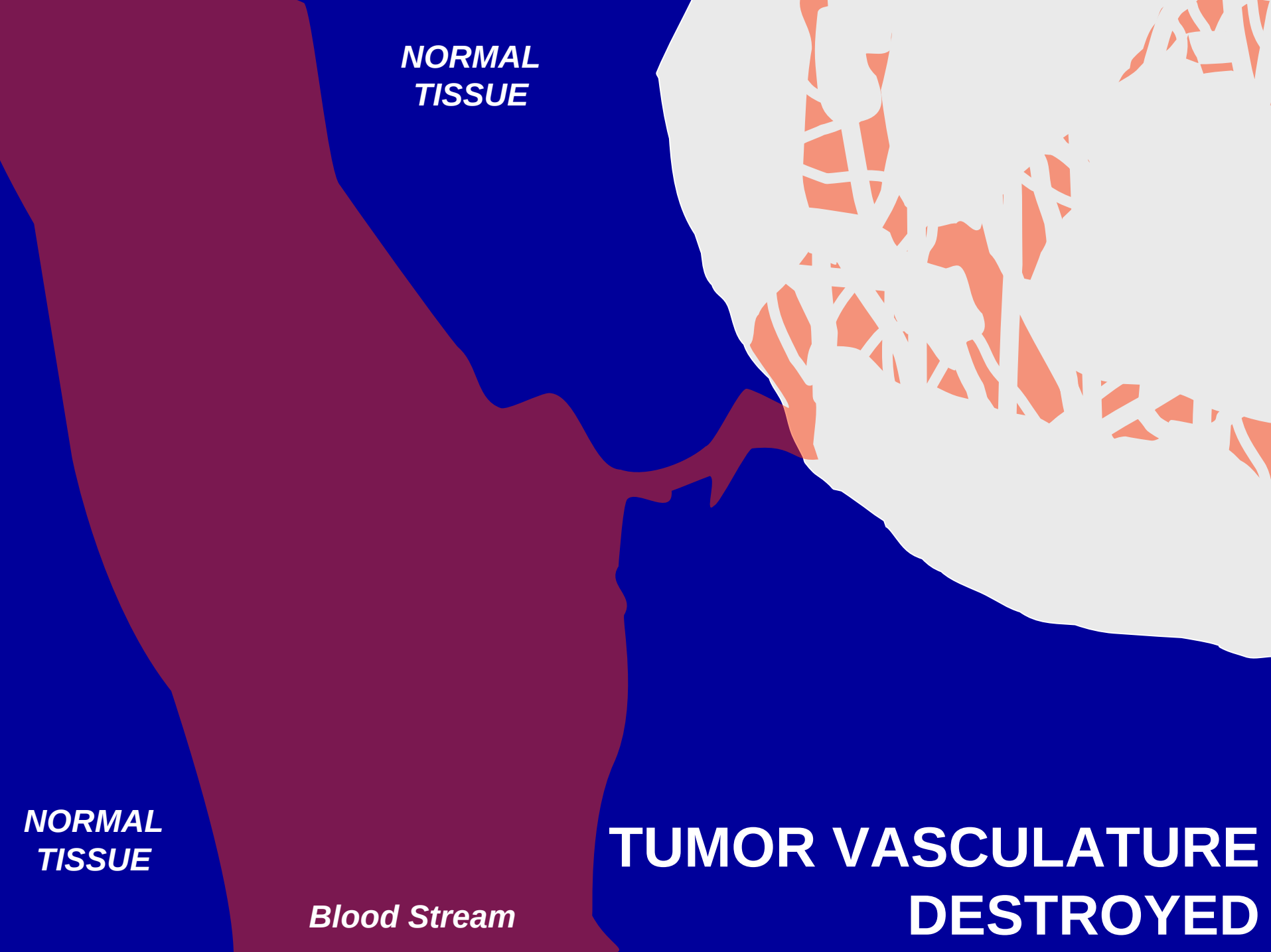

NORMAL TISSUE
TF
TF
TF
TF
TF
TF
TF
TF
TF
TF
TF
TUMOR
(CANCER)
NORMAL TISSUE
Blood Stream
PRE-TREATMENT

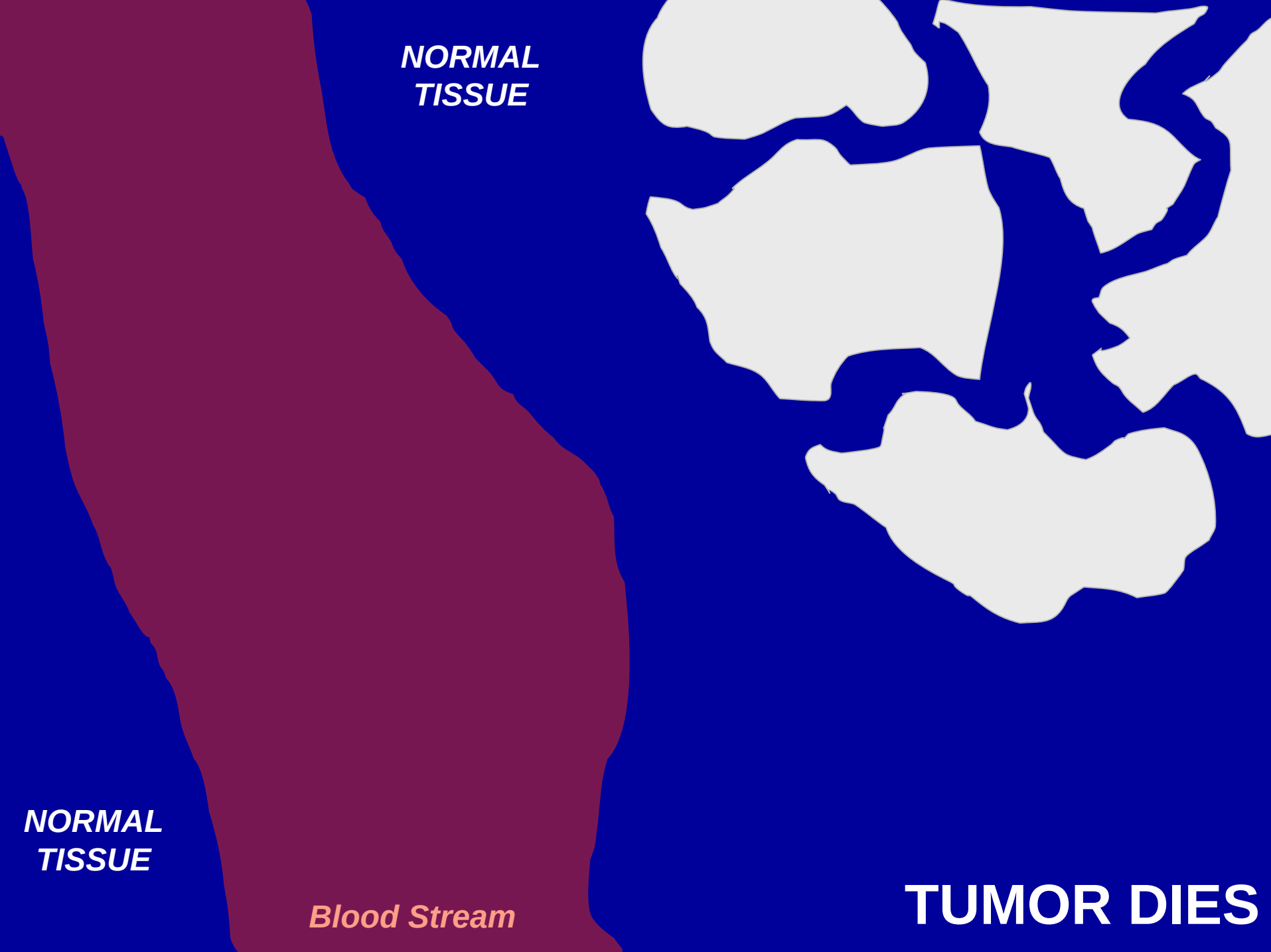

NORMAL TISSUE
TF
TF
TF
TF
TF
TF
TF
TF
TF
TF
TF
TUMOR
(CANCER)
NORMAL TISSUE
Blood Stream
INJECTION OF ICON


NORMAL TISSUE
TF
TF
TF
TF
TF
TF
TF
TF
TF
TF
TF
TUMOR
(CANCER)
NORMAL TISSUE
Blood Stream
BINDING OF ICON

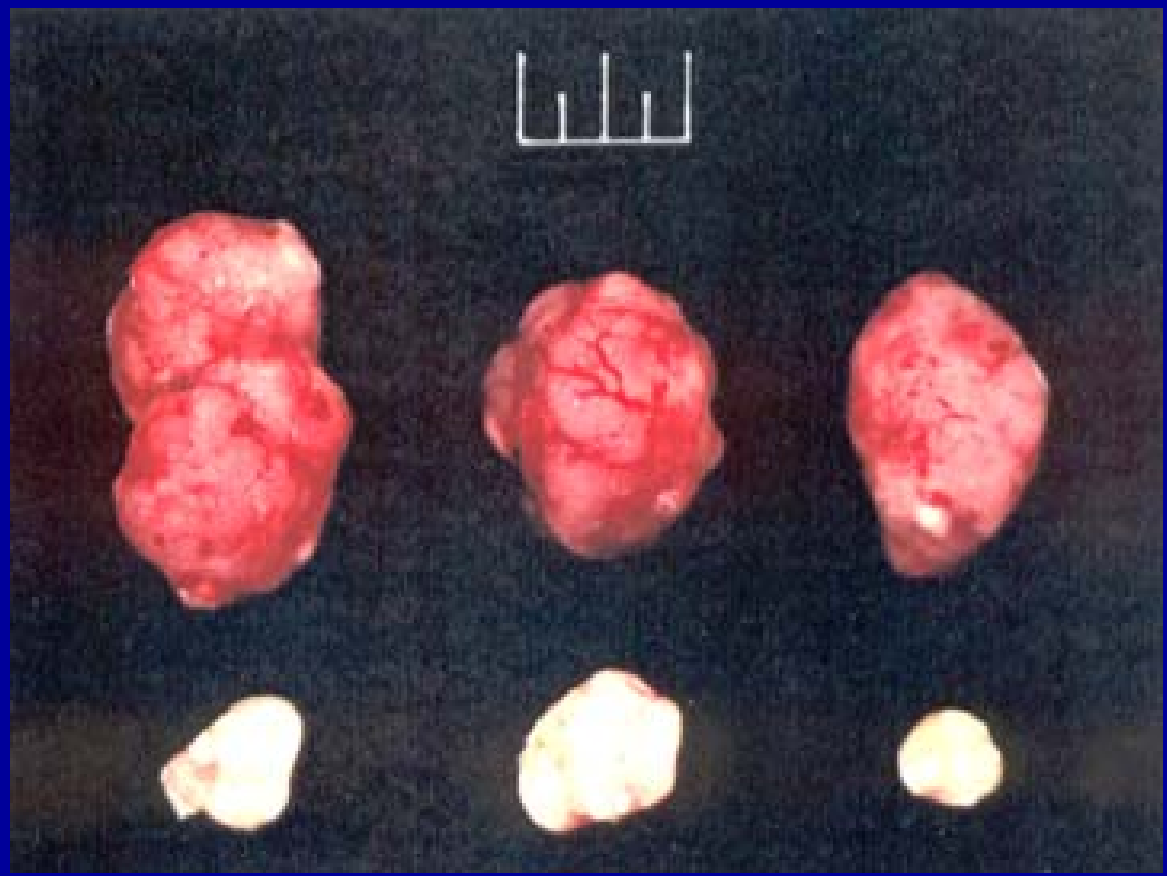

NORMAL TISSUE
TF
TF
TF
TF
TF
TF
TF
TF
TF
TF
TF
TF
TUMOR
(CANCER)
C
C
C
C
NORMAL TISSUE
Blood Stream
C
BOUND COMPLEMENT
Perforates EC


NORMAL TISSUE
NORMAL TISSUE
Blood Stream
TUMOR
(CANCER)
TF
NK
C
NK
NATURAL KILLER CELLS
Perforate EC

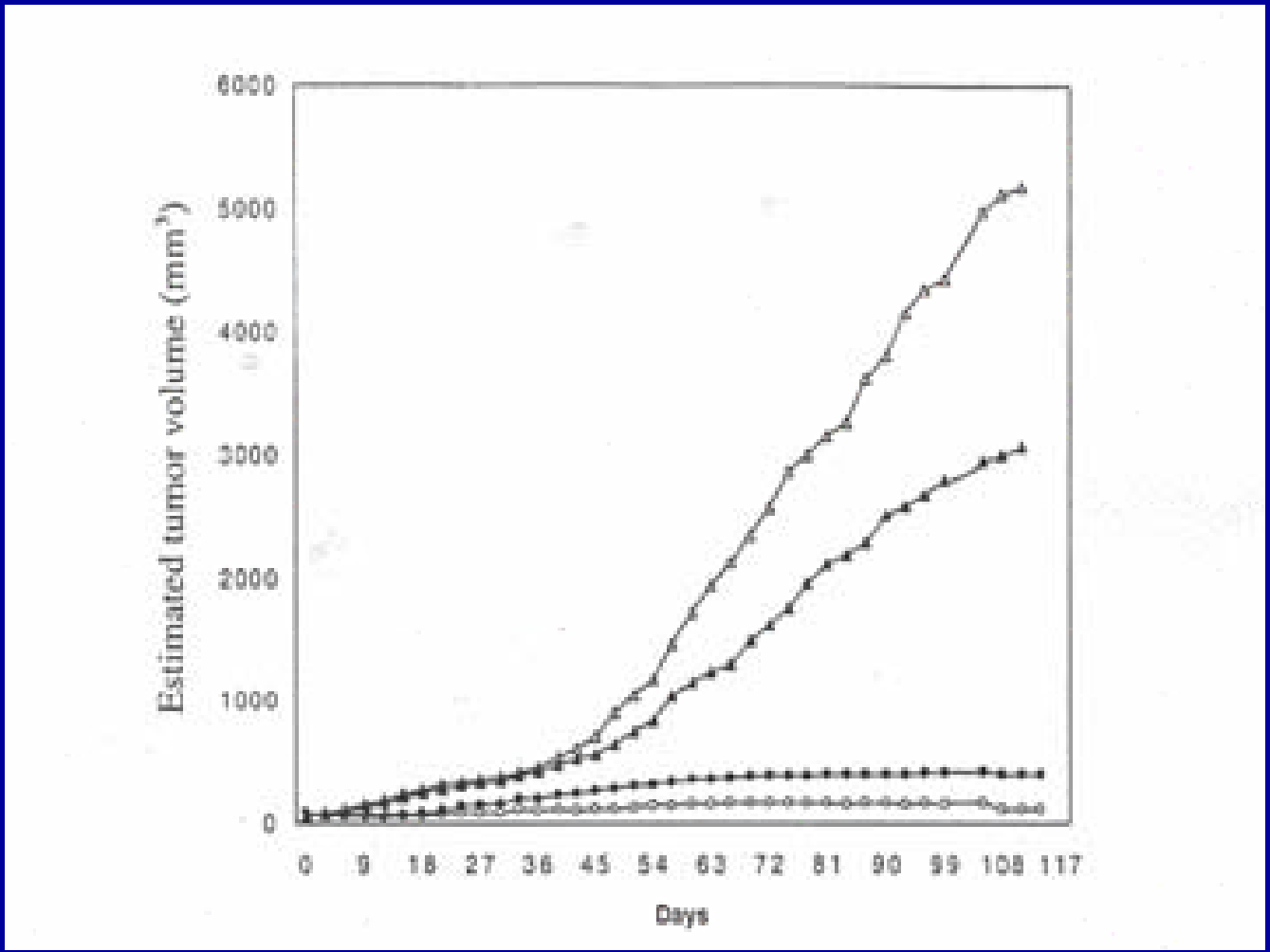

NORMAL TISSUE
NORMAL TISSUE
Blood Stream
TUMOR VASCULATURE DESTROYED


NORMAL TISSUE
NORMAL TISSUE
Blood Stream
TUMOR DIES

Icon Vector Induces Regressions of Subcutaneous Tumor Nodules in Human Tumor Xenograft Model



Intratumoral Injection of Icon Vector into Subcutaneous Tumor Nodules in Right Flank Suppresses Uninjected Left Flank Tumor



Proposed Phase I Clinical Trial of Intratumoral Injection of Icon Vector

- Intratumoral Injection of Icon Vector is Carried Out to Infect Tumor Cells at One Site. The Infected Cells then Secrete the Icon Protein Into the Bloodstream over a 10-14 day period. The Secreted Icon Protein Binds to Tumor Vasculature Throughout the Body and Induces Destruction of Tumor Vasculature and Tumor Cells

- Goal: To Assess Toxicity of Icon Vector Intratumoral Injections
- Study Design:
    a. 4 Dose Levels
    b. 3 Patients/Dose Level
    c. 12 Additional Patients at the Top Dose

- Toxicity Assessment
- Study of Binding of Icon, NK Cells, and Complement to Tumor Vasculature vs Normal Tissue Vasculature in Biopsies of Normal Skin and Tumor Nodule Before and After Icon Treatment To Establish “Optimal Dose” vs MTD
- Study of Response by Caliper Measurement of Subcutaneous Tumor Nodules and CAT Scans

History of Protocol Since December 2000

1. Presented to RAC on December, 2000.

2. Letter of Response to Questions of RAC on February

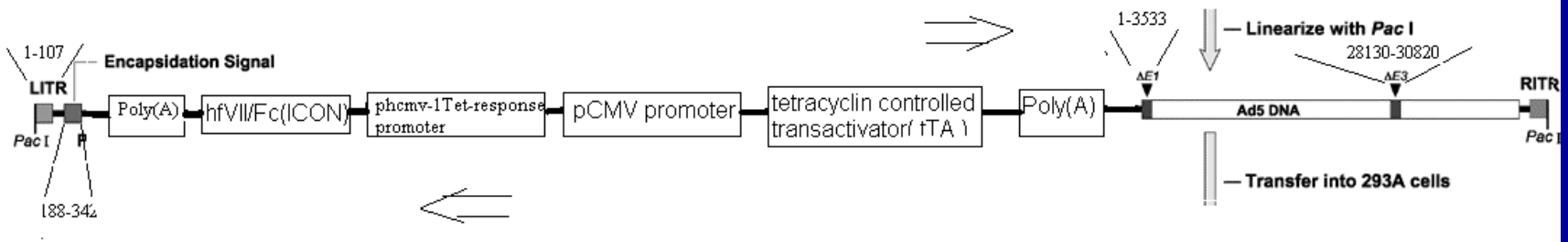
3. Moved from Yale to Sidney Kimmel Cancer Center, July, 2001

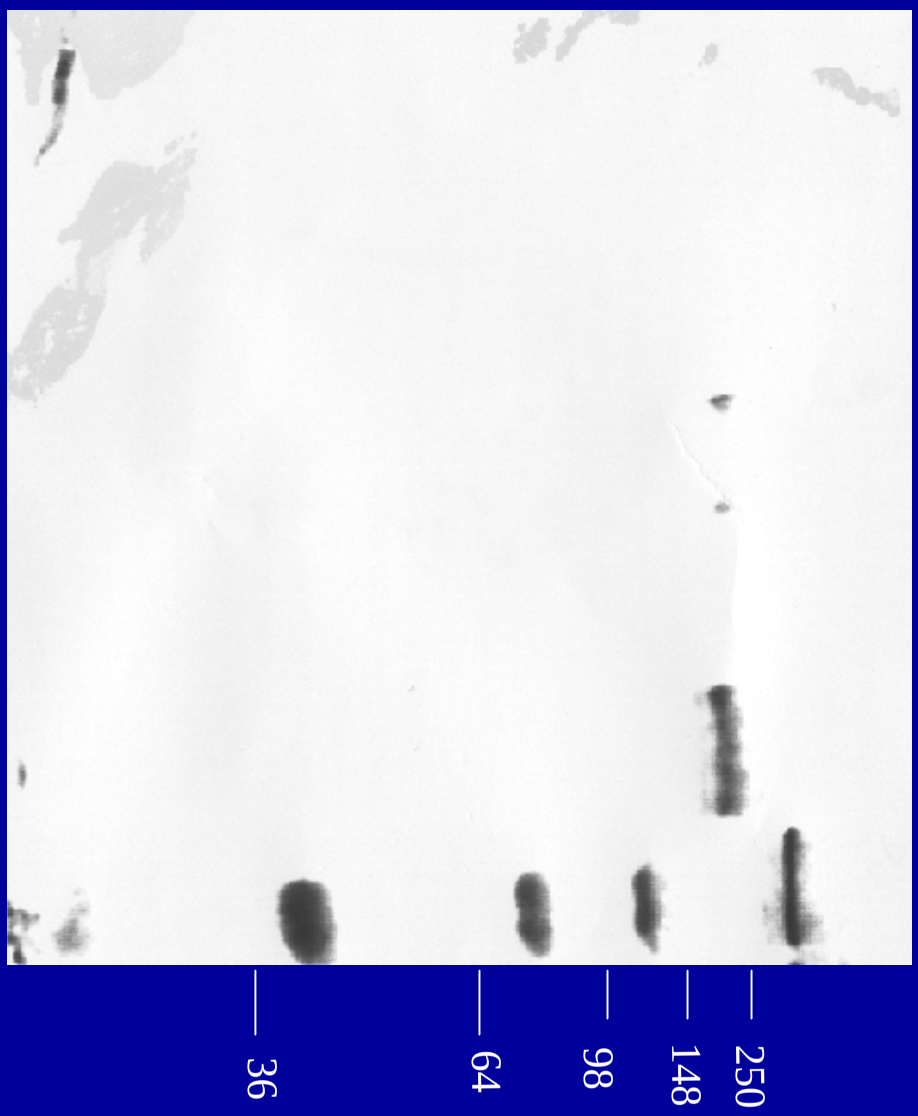
4. Three GMP Vector Preparations:
   a. Vector Preparation #1 Spring, 2001: Vector Contamination by GMP Contractor.
   b. Vector Preparation #2 Summer/Fall, 2002: Mold Contamination by GMP Contractor.
   c. Vector Preparation #3 Summer, Fall, Winter 2002: RCA and Low Yield. Data Suggested that Icon Suppressed Vector Yield.

5. Spring, 2003: Construction of Tet Inducible Icon Vector

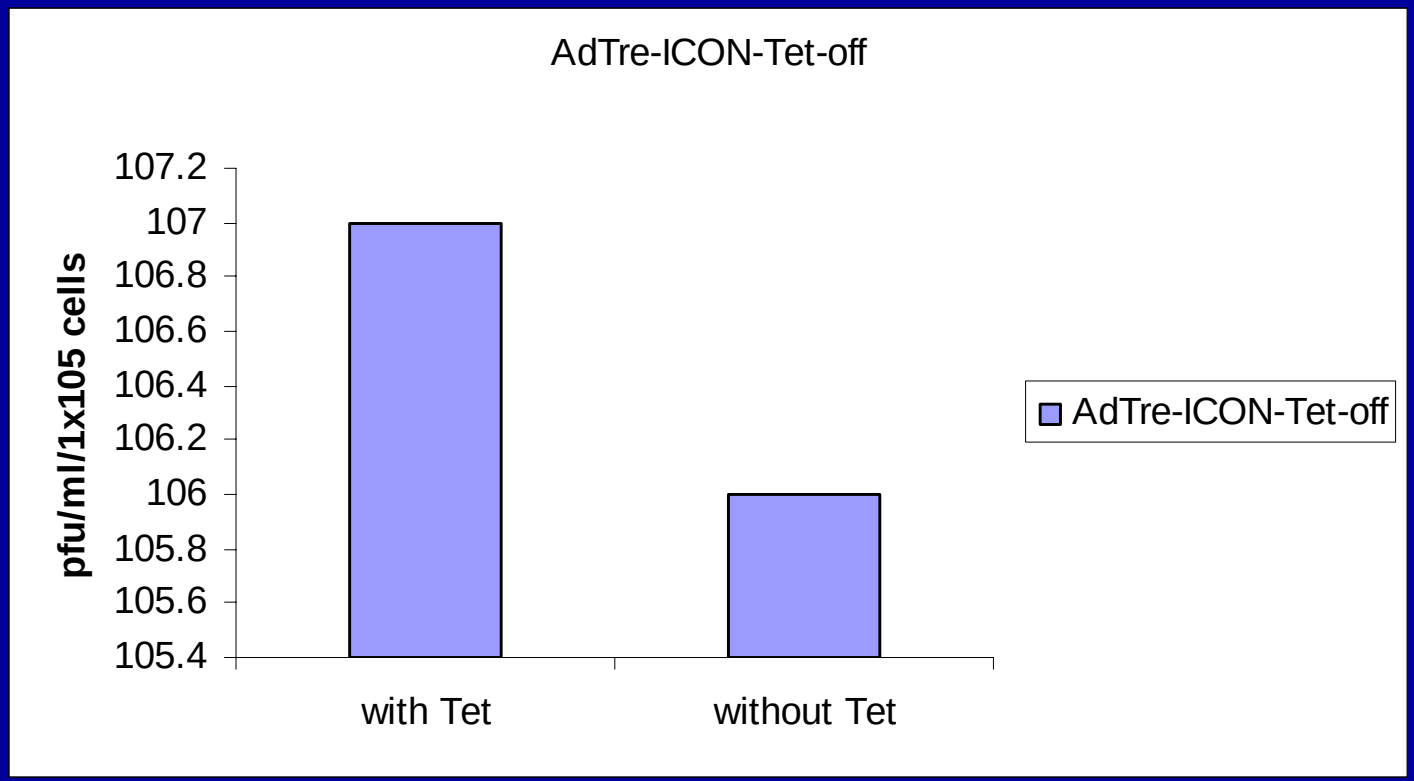
Measures Taken to Increase Yield and Eliminate RCA

1. Development of New Vector with Tet Inducible Icon Transcription Unit




293 control
AdI + Tet
AdI No Tet
36
64
98
148
250

Yield of Icon Vector with and without Tetracycline



2. Planning of GMP Production on PerC6 for Phase I Trial

PerC6 and the Tet Icon Vector have no Overlapping DNA Sequence in the Helper Function Casette. Therefore, the probability of RCA with PerC6 production is low (Fallaux et al, Human Gene Therapy 9: 1909, 1998.

Schedule of Proposed GMP Production

a. Transfection of PerC6 with Tet Icon Vector DNA (July, 2003). Yield is 50,000 IU/cell in presence of Tet.

b. 3 Sequential Plaque Purifications with Screens of Clones after 2nd and 3rd Plaque Purifications (Ongoing)

c. Amplification after Screening of Clones for Yield and Expression of Icon (Early October, 2003)

d. PerC6/Icon Vector GMP Production Lot in (October-November, 2003).

e. Quality Control Studies (December, 2003).

f. FDA IND Submission in January, 2004.

Explanation of Changes in Phase I Trial Clinical Protocol with Tet Icon Vector

The following changes in the protocol should result in increased patient safety, reduction of the risk of toxicity, and a more informative trial.

1. **The number of indications has been increased to include patients with carcinoma of the breast and head and neck, as well as sarcoma and malignant melanoma. This will allow study of a greater range of patients for toxicity and increase the rate of accrual.**

2. **The upper age limit of the patients has been reduced to 45 to lower the risk of undiagnosed cardiac disease being present.**

3. **The number of intratumoral injections of icon vector has been reduced from 6 to 3 (3 induces the maximal response).**

Continuation of Changes in Protocol (P.2.)

4. The dose of the top cohort has been reduced from $1x10^{13}$ to 4×10^{12} (this dose is the human equivalent to the effective dose in the pre-clinical model.

5. The clinic visit schedule for tumor measurement, physical exams and lab tests has been reduced to encourage patient compliance yet still maintains adequate surveillance.

6. The trial will now be conducted at the Sharp HealthCare Hospital System in San Diego CA instead of Yale University since the move of A. Deisseroth from New Haven to San Diego. Sharp HealthCare is affiliated with the Sidney Kimmel Cancer Center. The first patient will be treated in the hospital and if no significant toxicity occurs, the subsequent treatment will be given as out patients in an oncology clinic adjacent to the hospital.

Continuation of Changes in Protocol (P.3.)

7. We have eliminated some tests for the entrance criteria including the pulmonary function tests, bone scan and MUGA scan.

8. The entry and exclusion criteria are carefully defined with minimum and maximum values to reduce the risk for study participants.

9. Positron Emission Tomography (PET) and Dynamic MRI have been added at 2 time points in an effort to determine the optimal dose as well as the MTD.

10. The informed consent is HIPPA compliant and has been approved by the local IRB.

Follow up on Questions of RAC from January, 2001

Question # 1: Larger Animal or Second Model for Safety Testing.

Question # 2: Safety Testing on Actual hfVII/hIgGFc, not mfVII/IgGFc.
Problem: Need animal model with human Tissue Factor to bind the hfVII/hIgGFc.

Answer: Mouse Model of Nigel Mackman with knock-out of mouse Tissue Factor and insertion of human TF transgene, expressed at physiological levels (PNAS 96: 8138, 1999).

Safety Testing Experiment: Injection of hfVII/hIgGFc into tail vein of Mackman Mouse at 10X and 100X the dose necessary to produce level of icon proposed for the top dose of the Phase I clinical trial (Ongoing).

Follow up on Questions of RAC from January, 2001

Question # 3: Most of Pre-clinical data is in SCID mouse. Please carry out toxicity and efficacy in Immunocompetent Mouse Models

Answer:

- **Completed Toxicity and Efficacy Studies in Immunocompetent Mice for:**
- **Prostate Cancer (5 Test Mice and 5 Controls)**
  Melanoma (5 Test Mice and 5 Controls)
  EMT Breast Cancer in BalbC Mice (9 Test Mice and 9 Controls))

Result:

- **Efficacy seen in Prostate, Melanoma and Breast Models.**
- **No Toxicity seen in Prostate, Melanoma and Breast Models.**

Follow up on Questions of RAC from January, 2001

Question # 4: RAC suggested studies of potential immunogenicity of the icon in an immunocompetent mouse

Answer: Assay for neutralizing antibodies to the mfVII/mIgGFc)

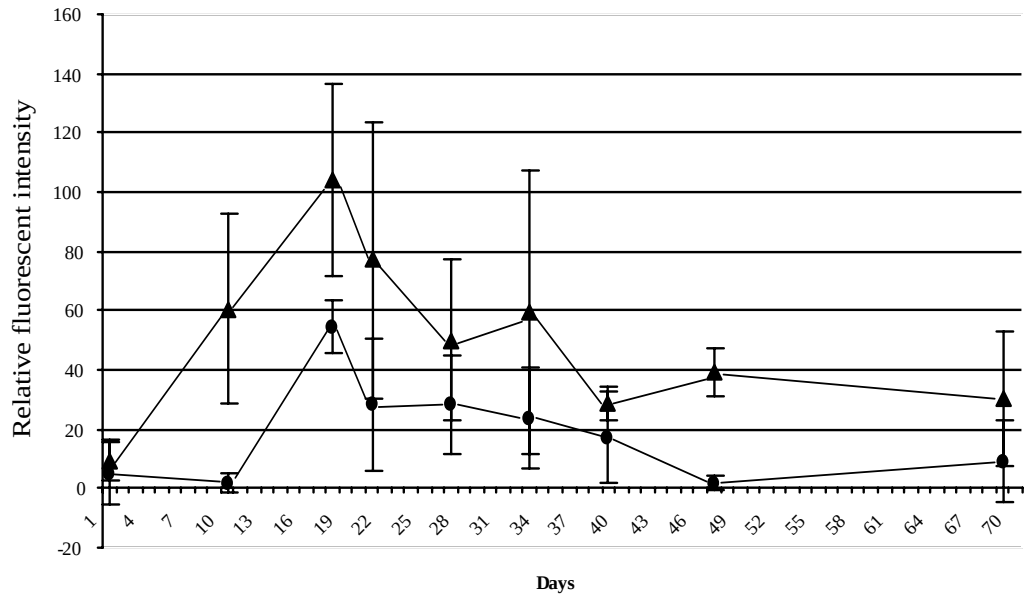
Antibody Assay Method:

1. **Coat Multiwell plate with icon protein;**
2. **Block with BSA;**
3. **Add diluted plasma samples from test mice;**
4. **Wash and Add Anti-mouse IgGFc;**
5. **Add FITC labeled horse anti-goat IgG;**
6. **Read Fluorescent Intensity.**

Result of Experiment:

Transient Low Level of Antibodies Detectable to mfVII which disappear by Day 20 following 6 vector injections given every 3 days.

No suppression of efficacy.



▲ : The titer of the mfVIImFc Icon in blood samples of 5 immunocompetent C57BL/6 mice.

○ : The titer of an antibody against the mfVII domain of the Icon in the same blood samples of the mice.

Follow up on Questions of RAC from January, 2001

Question # 7: RAC requests biodistribution data on mouse experiments to ascertain if there is any unintended icon expression outside the tumor target.

Proposed Biodistribution Experiment:
Subcutaneous Injection in nude mouse of 1 X 10^7 MDA MB 435 human breast cancer cells-grows up in 7 days to 50-200 cu mm: one site on the rear flank of each animal.

Route of Icon Vector Administration: intratumoral injection. Size of tumor nodule: 50-200 cu mm, volume of vector solution: 30% of tumor. Each day's injection given in 3 aliquots 5 minutes apart.

Number of injections on separate days; 3 total, every 3 days.
Dose: 1 X 10^9 viral particles (VP), 1 X 10^{10} VP, 2.5 X 10^{10} VP, and control (no viral particles). Top dose is at least 25 times the minimal effective dose.

Follow up on Questions of RAC from January, 2001

Continuation of Biodistribution Experiment (P.2.)

Number of animals/group: 5/group

Time Points for Autopsies: 3 days, 1 week, 3 weeks after the last injection.

Total number of animals: 4 doses X 5 mice/group X 3 time points=60 animals.

Observations: 3 times/week: record for presence and absence of: inactivity, diarrhea, pale mucous membranes, bleeding, hunching, ruffled fur.

Weigh: Weekly.

Continuation of Biodistribution Experiment (P.3.)

Autopsies on each animal. Collection of 5 organs (liver, kidney, lung, brain and heart) and fixation. Sections of all five organs will be placed on a single slide and stained with hematoxylin and eosin. A second slide will used for staining with an antibody for the icon.

Number of organs: tumor (injected), liver, kidney, brain, heart.
Tests on each Organs Slide: hematoxylin and eosin, icon, Q-PCR for vector level in each organ.

Total Number of Slides: 60 animals X 2 slides=120 slides; QPCR=60 samples.

Blood Tests: Collect at 24 hours, 1 week, and 3 weeks after the last injection.

Types of Blood Tests: ALT, Creatinine, Hemoglobin, Platelets, Prothrombin time, and QPCR for vector DNA.

Total Number of samples/animals: 4 doses X 3 time points X 5 animals/group= 60 samples/ test.

Number of tests/animal: 6 (excluding icon) X 60 animals=360.

Icon Levels By ELISA: 60 animals= 60 icon tests.

Pre-clinical Data Supporting Proposed Vector Trial

1.	Threshold of Toxicity (Prolongation of PT)	5 mcg/ml
2.	Serum level of Icon associated with response	50 ng/ml
3.	Minimum Vector Particle Dose Associated with Response	$7X10^{8}$VP/dose
4.	Optimal Vector Particle Dose For Response	$2 X 10^{9}$VP/dose
5.	Dose in Man Equivalent to Dose in Mice	$4 X 10^{12}$/dose

Schedule: Every Three Days (since tumor started to regrow in 3 days In xenograft model and vessels start to regrow in 3 days in IVM model). Thus, the vector injected every 3 days

Number of Injections is 3 since the maximal response achieved in Xenograft model by 3 doses

Retreatment provided since in xenograft model, retreatment after 2-3 weeks between first treatment series and second gives durable complete remissions in the mouse, whereas partial responses only with the first series.