Tumor Microenvironment Network (TMEN)

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TMEN 2006-2011: Goals

– *Generate a comprehensive understanding of the composition of the normal stroma and the role of the stroma in tumor initiation, progression and metastasis*

– *Develop resources and infrastructure critical to the broader research community to advance understanding of the tumor microenvironment (TME)*

TMEN 2006-2011: Current Program

- **Nine interdisciplinary groups characterizing the TME in major cancer sites, emphasizing human samples**
  - *Identifying and translating promising leads*
- **Collaboratively addressing the complexity of TME by assessing:**
  - *The co-evolution of tumor-associated stromal cell types and the tumor*
  - *Stromal cell interactions with each other and with tumor cells*
- **Generating novel reagents, models and technologies for the research community**

TMEN 2006-2011: Areas of Emphasis

Microenvironment

Initiation   **Progression**   **Metastasis**

- *Tumor initiating cells*
- *Tumor heterogeneity*
- *Stromal cell types and cellular processes*
- *Complex signaling pathways*
- *Genes and genetics*

TMEN 2006-2011: Scientific Accomplishments

Tumor Initiating Cells:

- *Combined invasive gene signatures in tumor initiating cells and stromal wound repair response genes robustly predict metastasis-free and overall survival in breast, medulloblastoma, lung and prostate cancer patients*

- *New therapeutic approaches to circumvent the chemo- and radio-resistance of tumor initiating cells*

TMEN 2006-2011: Scientific Accomplishments

Tumor Initiating Cells:

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- *New therapeutic approaches to circumvent the chemo- and radioresistance of tumor initiating cells*

Stromal Cells:

- *Delineation of the mechanisms by which bone marrow-derived cells affect tumor growth, invasion, inflammation, angiogenesis and metastasis*
- *Cancer-associated reactive stromal volume predicts progression to biochemical recurrence in human prostate cancer*
- *Cancer-associated neurogenesis in human prostate predicts aggressive disease*
- *Tumor microenvironment signature of metastasis (TMEM), which includes tumor cells, TAMs and endothelial cells, accurately predicts systemic metastasis from primary breast cancer samples*

TMEN 2006-2011: Programmatic Accomplishments

Scientific Collaborations

- *Identification of human tumor initiating cell fate using intravital imaging*

- *Characterization of human prostate stromal changes, both genetic and epigenetic*

- *Development of imaging beacons to determine role of specific metalloproteases in tumor and stroma*

TMEN 2006-2011: Programmatic Accomplishments

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Critical Resources Developed

- *Validated EHS sarcoma matrix ("Matrigel") resource*

- *Stromal antibodies – validation of existing antibodies and development of novel antibodies for stromal and tumor initiating cells*

- *Transgenic mice expressing stromal TVB receptors for targeting with RCAS vectors*

- *Bank of tumor initiating cells derived from early passage human tumors of head and neck, breast, colorectal cancers*

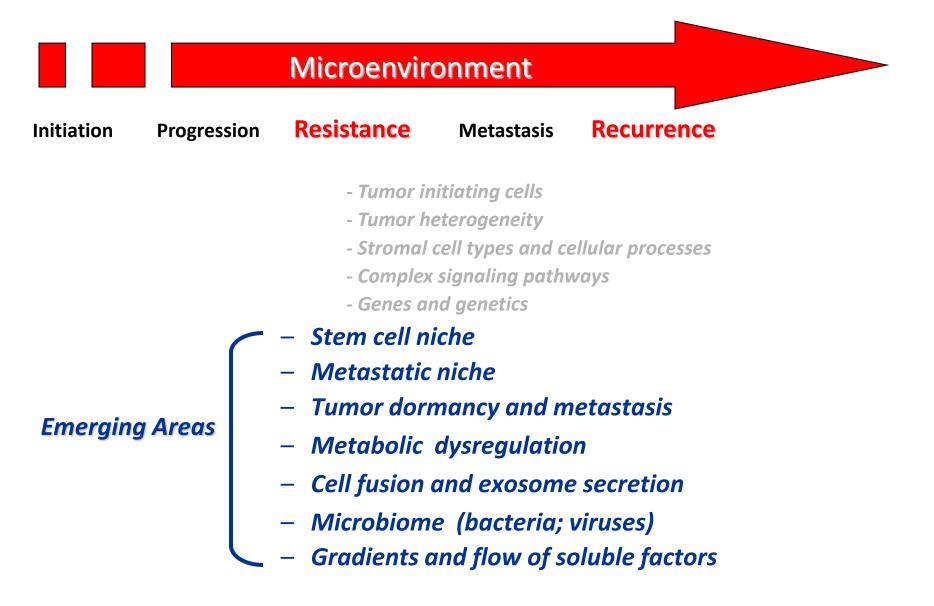- *Bank of murine bone marrow cells, for generating BMDC*

Proposed TMEN Reissuance Concept: <u>Why?</u>

- *Investigation of emerging novel concepts in TME will require new teams of interdisciplinary scientists to collaborate on large scale, complex problems*

- *Network structure will:*
  - *Accelerate the development of new areas in TME, and of resources for the research community. (Investigator-initiated mechanisms are not suited for such efforts.)*
  - *Provide a focus for interacting with other NCI programs, to promote interdisciplinary efforts*
  - *Encourage established investigators to study the TME, through the collaborative U01 program*
  - *Facilitate training of junior investigators*

TMEN Reissuance: Areas of Emphasis

Microenvironment

Initiation   **Progression**   **Resistance**   **Metastasis**   **Recurrence**

- *Tumor initiating cells*
- *Tumor heterogeneity*
- *Stromal cell types and cellular processes*
- *Complex signaling pathways*
- *Genes and genetics*

Emerging Areas

– *Stem cell niche*

– *Metastatic niche*

– *Tumor dormancy and metastasis*

– *Metabolic dysregulation*

– *Cell fusion and exosome secretion*

– *Microbiome (bacteria; viruses)*

– *Gradients and flow of soluble factors*

Proposed TMEN 2011-2016

- *Open RFA competition emphasizing emerging areas, soliciting new interdisciplinary teams*

- *Up to 9 Research Programs (U54 Consortia)*

- *Continued support at current total level of $9.08M/year*
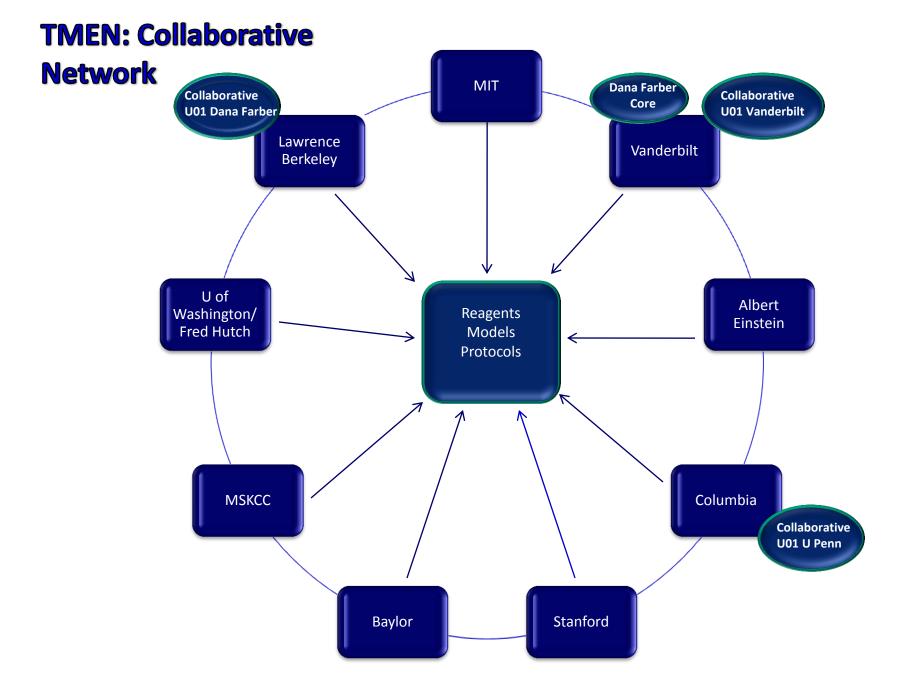
- *Five year duration*

Thank you!

Questions?

Background Information

TMEN Grant and Network Activities Funding FY2006-2010

TMEN Project and Network Activity	Average Total Cost per Year
Individual Grant Support 9 U54's(5 fully funded, 4 partially funded)	**$8,401,355**
Network Activities Resource development Collaborative projects	**$ 684,033**
Total Cost per year	**$9,085,388**

Current TMEN Principal Investigators

- **Mina Bissell (LBL, UC Berkeley)**
- **Michael Clarke (Stanford)**
- **John Condeelis (Albert Einstein)**
- **Eric Holland (Memorial Sloan Kettering CC)**
- **Richard Hynes (MIT)**
- **Lynn Matrisian (Vanderbilt)**
- **Stephen Plymate (Univ. Washington)**
- **David Rowley (Baylor)**
- **Timothy Wang (Columbia)**

TMEN: Collaborative Network

TMEN 2010-2015: Examples of Scientific Goals

Using multi-disciplinary approaches , delineate biological mechanism(s) governing the TME interactions which are critical for tumor development, progression and metastasis, and therapeutic resistance and tumor recurrence

- Focus on specific tumor sites, using human cancer tissues and models

- Characterize component cells and matrix molecules in normal organ and tumor-associated stroma; examine alterations in the microenvironment

- Identification of lineages of bone marrow-derived cells and immune cells and their effects in tumor initiation, progression and metastasis

- Identify and characterize the stem cell niche; origin of stromal cells

- Characterize the role of microbiome (bacteria and viruses)

- Characterize the role of metabolic dysregulation in TME

- Delineate the functional relevance of tumor cell-host cell fusion and exosomes

- Determine the effects of cancer therapy on the TME

- Identify and characterize the role of TME in therapeutic resistance

- Identification and characterization of gradients and flow of soluble factors

TMEN Evaluation Criteria

The following broad criteria will be used to judge the success and effectiveness of the proposed program

- Successful identification of novel components of the normal and tumor stroma in primary and metastatic tumors

- New insights into the composition, role and origin of tumor-associated stroma during cancer initiation, progression and metastasis

- Determining the effects of cancer therapy on the tumor microenvironment

- Consortium participation in collaborative activities aimed at improving existing technologies and developing novel reagents

- Development and effective dissemination of resources to the research community

Independent Program Evaluation Report

- Addressed goals of the original RFA

- Evaluation based on literature, NCI grants documentation, interviews and staff interaction

- Separate evaluations from outside experts

  - **Reviewers' comments reflect their overall enthusiastic endorsement**

- "Overwhelmed by the accomplishments of the groups associated with TMEN- both in terms of number of critical questions being addressed, the quality of the investigators and the innovative approaches being pursued."

- "if research in this area was funded entirely through the R01 mechanism, there would only be a trickle of isolated research projects in the area "

- "The coordination of resource generation has been largely driven by staff."

- "the program has exposed investigators who were working in select areas of the microenvironment to think 'bigger'

Current Portfolio Analysis

- TMEN is the only funded program addressing a comprehensive approach to understand the TME, and its critical role in mediating tumor progression, metastasis and therapeutic resistance

- The total TME portfolio budget in NCI is $43M. DCB's portfolio includes the current 9 TMEN U54s, 3 U01s, and 61 investigator-initiated grants

- Very few grants focus on the new areas proposed in this reissuance. Most examine cell migration, proteases, angiogenesis, hypoxia or metastasis

- Investigator-initiated grants are unable to pursue comprehensive studies to address the microenvironment using "tumor-as- an-organ" approach as these mechanisms are not suited for to these types of studies

Supplemental Resource Information

BMDC bank
Bone marrow cells will be isolated from two commonly used strains of mice. These are:
(a) the Rosa26 (C57BL/6Jtrosa26) mice
(b) smooth muscle a-actin promoter driving a reporter (EGFP)

RCAS Vector development targeting stromal cells
a) two RCAS(A) and two RCAS(B) vectors were made. Just to clarify, these are the avian virus vectors that infect the respective transgenic mice that express the RCAS (A or B) receptors called TVA or TVB. Two TVB and one TVA mouse model have been generated
(i) FSP1-TVB mice (S100A4, that is expressed in many cells of mesenchymal origin including fibroblasts).
(ii)SM22-TVB mice (expressed in smooth muscle cells).
Currently the founders are being characterized at Vanderbilt – they have already been initially screened by Lionel Feigelbaum (sp?)
b) The third mouse (started late in the contract) is the "hit and run" mouse that allows for expression of TVA upon crossing with any Cre mouse. That means one can target expressing either stromal or epithelial cell type.
 c) The RCAS are avian retroviruses that can only infect cells that express the appropriate viral receptor

Standardization of Antibodies and generation of novel antibodies

**Antibodies used for flow cytometry isolation of cells - sent to NCI repository
 for distribution to other TMEN laboratories and the broader NIH research community**

- FLOTILLIN-2/ESA FITC MAB 29
- HU CD10 PE-CY5 MAB HI10A
- HU CD140B BIOTIN MAB 28D4
- HU CD166(ALCAM) PE
- HU CD18 PE-CY5
- HU CD2 PE-CY5
- HU CD20 PE-CY5 MAB 2H7
- HU CD24 FITC MAB ML5
- HU CD24 PE MAB ML5
- HU CD3 PE-CY5 MAB UCHT1
- HU CD31 BIOTIN MAB M89D3
- HU CD31 BIOTIN MAB WM59
- HU CD44 APC MAB G44-26
- HU CD45 PE-CY5 MAB HI30
- HU CD45 PE-CY7 MAB HI30
- HU CD64 PE-CY5 MAB 10.1
- HU CD66 FITC
- HU CD66 PE
- HU EGFR FITC (0.05MG)
- HU EGFR PE
- MS CD140A BIOTIN MAB APA5
- MS CD90.1(THY1.1) APC
- MS H-2KD BIOTIN MAB SF1-1.1
- SAV PE-CY5
- SAV PE-CY7

List of cell lines generated for antibody target overexpression

PC12 parental cell line

PC12 vector control cell line

PC12 Laminin A4 overexpression cell line

293T lysates from overexpression of TGFβ1,β2 and β3 as well as Cercam and Leprel 2 have been generated and used for Elisa and western blot screening of antibodies.

List of stable knockdown cell lines for antibody targets

MCF7 GFP shRNA KD cell line

MCF7 TGFβ1 KD cell lines

MCF7 TGFβ2 KD cell lines

MCF7 TGFβ3 KD cell lines

MDA435 GFP KD cell line

MDA435 Leprel 2 KD cell lines

MCF7 CerCam KD cell lines and GFP KD control line

T98G GFP KD cell line

T98G Laminin Alpha 4 KD cell lines

Antibodies

mAb against Cercam

mAb against Laminin Alpha 4

antibody supes against TGF β1/2/3 as well as Leprel 2

REAGENTS AND PROTOCOLS

Protocol for separation of mouse stroma from human tumor cells

Protocol for separation of human epithelial tumor cells from non-tumor components

Protocol for LCM following by high-resolution oligo array-CGH

Protocol for optimized WGA

Custom Agilent CGH 2x415K oligo microarray, optimized for resolution vs. cost

Monoclonal Antibodies against Laminin α4 and Cercam

Tumor initiating cells derived from early-passage human xenograft cancer tumors established from cells isolated directly from patient tumors - sent to the NCI.

Colon cancer - 9 xenograft tumors

Breast cancer - 5 xenograft tumor

Head and neck cancer - 10 xenograft tumors

TMEN Publications: 2006-2010

Year	Number of Publications
2010	18
2009	101
2008	64
2007	43
2006	6
In prep (2010)	9
Total	232