Colon Cancer Vaccines

Michael A. Morse, MD, MHS, FACP
Professor of Medicine
GI Oncology
Duke University Medical Center
Disclosures

• Grant support: Prometheus, Aduro, Etubics, AlphaVax, BMS, IVT, Precision Biologics, Newlink

• Consultant: Amgen, BMS, Genentech, Bayer/Onyx, Sanofi, Regeneron

• Speaker: Prometheus, Genomic Health, Genentech, Novartis, Celgene, Bayer/Onyx
Objectives

• Describe the mechanism of action of vaccines for colon cancer
• Discuss current status of clinical trials for colon cancer vaccines
• Discuss how data generated from ongoing studies is influencing future developments in colon cancer vaccines
T cells recognize and destroy colon cancer

<table>
<thead>
<tr>
<th>Cells</th>
<th>CD8(^+)CD45RO(^+) T cells</th>
<th>(T_H,1) cells</th>
<th>(T_H,2) cells</th>
<th>(T_H,17) cells</th>
<th>(T_{\text{Reg}}) cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>Good(^{108-106})</td>
<td></td>
<td></td>
<td></td>
<td>None(^{21,15})</td>
</tr>
<tr>
<td>Head and neck cancers</td>
<td>Good(^{106,109,110})</td>
<td></td>
<td></td>
<td>None(^{29})</td>
<td>Good(^{119,120})</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Good(^{111-114})</td>
<td><em>Good(^{111,115})</em></td>
<td><em>Good(^{41})</em></td>
<td></td>
<td>None(^{29})</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>Good(^{118,119})</td>
<td></td>
<td></td>
<td></td>
<td><em>None(^{24})</em></td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Good(^{120-122})</td>
<td>Good(^{119,124})</td>
<td>Poor(^{123})</td>
<td>Good(^{125})</td>
<td><em>None(^{16})</em></td>
</tr>
<tr>
<td>Oesophageal cancer</td>
<td>Good(^{120,127})</td>
<td>Good(^{120})</td>
<td></td>
<td></td>
<td>Good(^{129})</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Good(^{143,75,91,16,61,70,110-145})</td>
<td>Good(^{56,70})</td>
<td>None(^{96})</td>
<td>Poor(^{136,140})</td>
<td><em>None(^{13})</em></td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td><em>Good(^{11})</em></td>
<td>Good(^{71})</td>
<td></td>
<td></td>
<td>Poor(^{110})</td>
</tr>
<tr>
<td>Prostatic adenocarcinoma</td>
<td>Good(^{151-155})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung carcinoma</td>
<td><em>Good(^{11,124-137})</em></td>
<td>Good(^{151})</td>
<td></td>
<td>Poor(^{156})</td>
<td>Poor(^{160-162})</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>Good(^{169})</td>
<td>Poor(^{164,165})</td>
<td></td>
<td></td>
<td>Poor(^{115})</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>Good(^{166})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anal squamous cell carcinoma</td>
<td></td>
<td></td>
<td></td>
<td>None(^{41})</td>
<td></td>
</tr>
<tr>
<td>Brain cancer</td>
<td></td>
<td></td>
<td></td>
<td>None(^{12,24})</td>
<td></td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td><em>Good(^{147,164})</em></td>
<td>Good(^{169})</td>
<td></td>
<td>Poor(^{176})</td>
<td>Poor(^{19,20})</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>Good(^{171})</td>
<td>Poor(^{171})</td>
<td></td>
<td>Good(^{172})</td>
<td></td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>Good(^{13})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Merkel cell carcinoma</td>
<td>Good(^{174})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urothelial cell carcinoma</td>
<td>Good(^{119})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular lymphoma and</td>
<td></td>
<td></td>
<td></td>
<td>Good(^{18})</td>
<td><em>Good(^{31,37})</em></td>
</tr>
<tr>
<td>Hodgkin's lymphoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>None(^{27})</em></td>
</tr>
</tbody>
</table>

\(T_H\), T helper; \(T_{\text{Reg}}\), regulatory T cell.
How to go from A to B?

Early Recurrence

No Recurrence
WHAT HAVE WE LEARNED
FROM CRC VACCINES
## Successes for active specific immunotherapy

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of Cancer</th>
<th>Treatment</th>
<th>Comparator</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hoover, 1993</td>
<td>Resected Colorectal Ca</td>
<td>Auto-tumor + BCG vs observation</td>
<td>Observation</td>
<td>Better DFS for colon but not rectal CA</td>
</tr>
<tr>
<td>Vermorken, 1999</td>
<td>Resected Colon Ca</td>
<td>Auto-tumor + BCG vs observation</td>
<td>Observation</td>
<td>Better RFS for stage II, but not stage III</td>
</tr>
</tbody>
</table>
Low clinical activity despite immunogenicity

• Review of 32 phase I/II studies including 527 patients with advanced or metastatic colon cancer (Clin Ca Res 2006;12:3064)
  – CR+PR: 0.9%
  – Clinical benefit (CR+PR+MR+SD): 11.2%

BUT:
59% humoral immune response
44% cellular immune response
Understanding the disconnect in results

Hope for the future
Is there an ideal antigen

- CEA
- MUC-1
- B-HCG
- EpCAM
- p53
- Gastrin
- 5T4

- Survivin
- SART3
Types of tumor antigens

- **Tumor specific**
  - Unique to tumor
  - essential for tumorigenesis/cancer progression
  - caused by somatic mutation
  - not found on any normal adult somatic tissues
  - Personalized

- **Tumor-associated**
  - appear on various cancer and normal cells, though with different expression levels.
NCI prioritization of cancer antigens

- therapeutic function
- Immunogenicity
- specificity
- oncogenicity
- expression level
- percentage of positive cells
- stem cell expression
- # of patients with antigen + cancers
- number of epitopes
- cellular location of expression

Cancer antigen pilot prioritization: representation of ranking based on predefined and preweighted criteria and subcriteria.

HER3 as a target

Aprile, Molecular Oncology 2013 Dec 25;1(1):7
Vaccinate with Ad-HER3

Isootype Control
CT.001
4%

VIA-LacZ
VIA-LacZ.002
8%

VIA-HER3
VIA-HER3.006
99%

Anti-HER3 MAb
anti-HER3.005
99%

Test serum for Abs that bind HER3
Fold Dilution

Mean Fluorescence Intensity (MFI)

-BT474M1 HER3-VIA
- BT474M1 GFP-VIA
- BT474 HER3-VIA
- BT474 GFP-VIA
- SKBR3 HER3-VIA
- SKBR3 GFP-VIA
- T47D HER3-VIA
- T47D GFP-VIA
- MDA-231 HER3-VIA
- MDA-231 GFP-VIA
<table>
<thead>
<tr>
<th>Epitope(s) position</th>
<th>Protein region</th>
</tr>
</thead>
<tbody>
<tr>
<td>101-111</td>
<td>ECD</td>
</tr>
<tr>
<td>153-167</td>
<td>ECD</td>
</tr>
<tr>
<td>185-191</td>
<td>ECD</td>
</tr>
<tr>
<td>209-223</td>
<td>ECD</td>
</tr>
<tr>
<td>369-375</td>
<td>ECD</td>
</tr>
<tr>
<td>501-511</td>
<td>ECD</td>
</tr>
<tr>
<td>589-599</td>
<td>ECD</td>
</tr>
<tr>
<td>649-663</td>
<td>TM</td>
</tr>
<tr>
<td>681-691</td>
<td>ICD</td>
</tr>
<tr>
<td>865-875</td>
<td>ICD</td>
</tr>
<tr>
<td>881-895</td>
<td>ICD</td>
</tr>
<tr>
<td>901-915</td>
<td>ICD</td>
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<tr>
<td>981-988</td>
<td>ICD</td>
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<tr>
<td>1037-1051</td>
<td>ICD</td>
</tr>
<tr>
<td>1105-1119</td>
<td>ICD</td>
</tr>
<tr>
<td>1153-1163</td>
<td>ICD</td>
</tr>
<tr>
<td>1249-1255</td>
<td>ICD</td>
</tr>
<tr>
<td>1268-1279</td>
<td>ICD</td>
</tr>
</tbody>
</table>
Ad-HER3 Vaccine-induced Antibodies (VIA) Lyse HER3+ Mammary Tumors by Complement Dependent Cytotoxicity (CDC)
Anti-proliferative effects
Internalization of HER3 after binding anti-HER3 antibodies

SKBR3

BT474M1

GFP-VIA

HER3-VIA
Antitumor activity of anti-HER3 antibody serum

![Graph showing tumor volume over days for AdGFP-VIA and AdHER3-VIA.](image)
Downstream signaling inhibition

- HER3-VIA
- GFP-VIA

- pTyr
- ErbB2
- ErbBr3
- Actin

1 2 3 4 5 1 2 3 4 5
Survival pathway downregulation

HER3-VIA  GFP-VIA

AKT

pAKT473

s6

ps6

Actin

1  2  3  4  5  1  2  3  4  5
Figure: CT26-hHER3 tumor growth in BALB/c mice treated with Ad-hHER3 vaccine (prevention model)
Escape: Downregulation of tumor antigen expression

PBS treated mice  GFP-VIA-treated Mouse tumor  HER3-VIA-treated Mouse tumor
Targeting naturally expressed epitopes relevant for cell survival
Antigens expressed within MHC molecules

BAP31

Rep Prot A

integrin b8 subunit precursor

cyclin I

International Immunology 2003;15:751-763
Vaccines: Does route affect outcome?

**Table 3. Influence of vaccination route, post hoc explorative analysis**

<table>
<thead>
<tr>
<th>Route</th>
<th>Rate CR, PR, MR, and SD</th>
<th>Humoral response</th>
<th>Cellular response</th>
</tr>
</thead>
<tbody>
<tr>
<td>s.c.</td>
<td>11% (11/99)</td>
<td>48% (31/64)</td>
<td>46% (32/70)</td>
</tr>
<tr>
<td>i.v.</td>
<td>14% (5/35)</td>
<td>ND</td>
<td>50% (7/14)</td>
</tr>
<tr>
<td>i.m.</td>
<td>12% (15/125)</td>
<td>60% (57/94)</td>
<td>29% (12/41)</td>
</tr>
<tr>
<td>i.d.</td>
<td>10% (16/156)</td>
<td>74% (34/46)</td>
<td>54% (36/67)</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.
Does type of vaccine influence outcome?

Table 2. Influence of vaccine, post hoc explorative analysis

<table>
<thead>
<tr>
<th>Vaccine Type</th>
<th>Rate CR, PR, MR, and SD</th>
<th>Humoral response</th>
<th>Cellular response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous tumor</td>
<td>46% (21/46)</td>
<td>(0/4)</td>
<td>(2/6)</td>
</tr>
<tr>
<td>Dendritic cell based</td>
<td>17% (12/70)</td>
<td>ND</td>
<td>53% (20/38)</td>
</tr>
<tr>
<td>Peptide</td>
<td>13% (9/69)</td>
<td>29% (7/24)</td>
<td>33% (19/58)</td>
</tr>
<tr>
<td>Idiotype antibody</td>
<td>3% (3/80)</td>
<td>69% (46/67)</td>
<td>53% (19/36)</td>
</tr>
<tr>
<td>Virus based</td>
<td>3% (4/135)</td>
<td>(4/4)</td>
<td>37% (20/54)</td>
</tr>
<tr>
<td>Other</td>
<td>8% (10/127)</td>
<td>61% (64/105)</td>
<td>52% (26/50)</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.
Dendritic cell vaccines

Uptake of genetic material, generation of protein, Processing and presentation of DC surface within MHC molecules
DC infected with poxvectors express antigen of interest

MOI 0

MOI 1.0

MOI 5.0

MOI 20

CEA

CD80
Phase I study of DC loaded with rF-CEA(6D)-TRICOM in patients with CEA expressing malignancies
Clinical trial strategies for DC vaccines

<table>
<thead>
<tr>
<th>Study Week</th>
<th>S</th>
<th>-1</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>10</th>
<th>14</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screen</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Leukapheresis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>DC generation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Administration of DC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeat pheresis</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Direct comparison of pre- and post-immunization blood samples

2nd cycle
ELispot result correlates with clinical outcome

SD/MR
172 +/- 19
P = 0.08
PD
66 +/- 8

Mean IFNg spots/100K cells

Baseline fp-TRICOM-CEA
Maximum Response for WT-Fowlpox
Maximum Response for fp-TRICOM-CEA
Composite IFN-gamma CFC in PBMC after vaccination
Minor clinical response following immunizations

Periaortic lymphadenopathy

Pre-immunization
Serum CEA= 46

4 months post-immunization
+ IFN alpha
Serum CEA= 6.8
Finding the best immune modulators

Phase I Study of Sequential Vaccinations With Fowlpox-CEA(6D)-TRICOM Alone and Sequentially With Vaccinia-CEA(6D)-TRICOM, With and Without GMCSF in Patients With Carcinoembryonic Antigen–Expressing Carcinomas

Fig 1. Overall survival by cohorts with or without granulocyte-macrophage colony-stimulating factor (GM-CSF). Cohorts 3 and 6 (16 of 19 patients experienced treatment failure) received no GM-CSF with vaccines; and cohorts 7 and 8 (12 of 24 patients experienced treatment failure) received vaccines plus GM-CSF.
Ad-IL-12 enhancement of vaccine

Osada ... Morse..et al., Cancer Immunol Immunother 2012
Mechanism of tumor vaccines

Mosolits, *Annals of Oncology, Online*
Cytolytic assay

NK Activity (against K562 cells)

Clinical Outcome

NK Activity (against K562 cells)

LPA 8%

LPB 24%

SD

LPC 47%

LPC 26%

LPB 12%

LPA 4%

SD

long period

LPA 54%

LPB 36%

PD

fp-TRICOM-21

fp-TRICOM-22

fp-TRICOM-07
Modulating the immune response once activated
Regulatory T cells versus antigen-specific T cell response

**Graph 1:**
- **Y-axis:** Percentage of lymphocytes
- **X-axis:** Time points (LPA, week 3, LPB, week 14, LPC)

**Graph 2:**
- **Y-axis:** Percentage of lymphocytes
- **X-axis:** Time points (LPA, week 3, LPB, week 14, LPC)

**Legend:**
- **CD4+CD25+ as % lymphocytes**
- **CD8+Tricom-CEA**
- **CD8+ CEA+ T cells**
- **CD4+ CEA+ T cells**
The image contains two graphs illustrating data related to the CD4+CD25+ expression levels and their impact on FoxP3 levels. The graphs show the distribution of relative FoxP3 levels and CPM (counts per million) across different CD4+CD25+ subsets.

**Graph 1 (Top):**
- The x-axis represents the CD4 expression levels, with CD4+CD25+Hi, CD4+CD25+Med, and CD4+CD25- subsets.
- The y-axis represents the relative FoxP3 levels.

**Graph 2 (Bottom):**
- The x-axis represents the CPM levels for CD4+CD25-, CD4+CD25Hi, and CD4+CD25Hi +CD4+CD25- subsets.
Regulatory T cells in colon cancer

Percent CD4+CD25+ Cells

- Normal Donors: 3.57
- All Cancer: 5.93
- Rectal: 6.96
- Colon: 5.95
- Metastasis: 6.95
Eliminating Regulatory T cells

Pre-ONTAK | Post-ONTAK

Pt. 1

Pt. 2

CD25
CD4
ONTAK + DC + rF-CEA(6D)-TRICOM

- Cohort 1: ONTAK x 1 followed by DC + rF-CEA(6D)-TRICOM
- Cohort 2: ONTAK followed by DC + rF-CEA(6D)-TRICOM x 4
Mean Number of Spots per 100,000 PBMC

Cohort 0

Cohort 1

Cohort 2
Cohort 0
Cohort 1
Cohort 2

Mean Percent IFNγ+
Vaccines that induce responses despite Treg

Alphavirus encoding CEA

Morse, J Clin Invest. 2010 Sep;120(9):3234-41
Immunity despite high Treg

Morse, J Clin Invest. 2010 Sep;120(9):3234-41
Developing vectors that evade neutralizing host responses

Ad5  
\[\text{Ad}[E1-] \quad \Delta E1 \quad \text{Transgene} \quad E2b \quad E2a \Delta E3 \quad E4\]

\[\text{Ad}[E1-,E2b-] \quad \Delta E1 \quad \Delta E2b \quad E2a \Delta E3 \quad E4\]

\[\text{Ad}[E1+] \quad E1aE1b \quad E2b \quad E2a \Delta E3 \quad E4\]
Induction of immunity despite pre-existing neutralizing titers

Number of prior Ad-WT injection(s) | 0 | 1 | 3 | 0 | 1 | 3 | 0 | 1 | 3
---|---|---|---|---|---|---|---|---|---
Ad[E1+] | | | | | | | | | | | 600
Ad[E1-] | | | | | | | | | | | 500
Ad[E1-,E2b-] | | | | | | | | | | | 400

IFN-γ positive spots/250,000 cells
Immunity despite pre-existing antivector immunity

Morse, Cancer Immunol Immunother 2013
Survival outcome
Utilizing colon cancer vaccines in standard clinical scenarios
VEGF effects on DC differentiation

DC (R1 and R2)

Undiff. cell (R1 and R4)
Correlation of VEGF and undifferentiated cells

% undifferentiated cells of the CD45+ cells in the cancer patients (0.50 +/- 0.31%) greater than healthy volunteers (0.32 +/- 0.16%) (p=0.011).
Figure 5

A

B

Percentages of Lin-DR+ cells (%)

0.47 0.53

time point

Pre  post

Percentages of Lin-DR- cells (%)

0.39 0.27 average

time point

Pre  post

Avastin-02 Avastin-03
Avastin-04 Avastin-05
Avastin-06 Avastin-07
Avastin-08 Avastin-09
Avastin-11 Avastin-12
Avastin-13 Avastin-14
Avastin-15 Avastin-16
B

**Figure B**

- **Graph**
  - **Y-axis**: 3H Thymidine Uptake
  - **X-axis**: Responder:Stimulator Ratio (1:1, 1:2, 1:4)
  - **Legend**:
    - `pre`
    - `post`

C

**Figure C**

- **Graph**
  - **Y-axis**: Stimulation Index Ratio (Post/Pre)
  - **X-axis**: Antigen
  - **Legend**:
    - Avastin-02
    - Avastin-03
    - Avastin-06
    - Avastin-07
    - Avastin-09
    - Avastin-11
    - Avastin-12
    - Avastin-13
    - Avastin-14
    - Avastin-15
    - Avastin-16
    - Avastin-17
    - Avastin-18
    - Avastin-19
## Trend for improved immune response to VRP-CEA in bevacizumab treated patients

<table>
<thead>
<tr>
<th></th>
<th>+ Bev</th>
<th>- Bev</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA+ CD4+ T cell by CFC</td>
<td>1.38%</td>
<td>0.49%</td>
</tr>
<tr>
<td></td>
<td>(P=0.059)</td>
<td>(P=0.13)</td>
</tr>
<tr>
<td>Anti-CEA Ab (Geo mean)</td>
<td>283</td>
<td>87</td>
</tr>
</tbody>
</table>
Vaccines can be given with chemotherapy: MVA-5T4 (Trovax™) + chemotherapy

Stage IV CRC → Trovax x 2 → Trovax x 2 → Trovax x 2

FOLFOX or FOLFIRI
X 12 cycles

Harrop et al., ASCO 2006, #2527
Immune response not impaired by chemotherapy

- 100% of patients had immune response in either ELISpot, proliferation, or Ab studies
  - Antibody titer higher than for TROVAX alone
- Similar immune response for FOLFOX or FOLFIRI
- Magnitude of immune response to 5T4 (but not MVA) correlated with clinical outcome
Clinical outcome compares favorably with chemotherapy alone

<table>
<thead>
<tr>
<th></th>
<th>FOLFOX + Trovax</th>
<th>FOLFIRI + Trovax</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR</td>
<td>73%</td>
<td>50%</td>
</tr>
<tr>
<td>ITT</td>
<td>68 weeks</td>
<td>67 weeks</td>
</tr>
<tr>
<td>mITT ≥ 2 immuniz</td>
<td>82 weeks</td>
<td>77 weeks</td>
</tr>
</tbody>
</table>
Should studies be done in NED patients?

Should survival be the endpoint rather than RFS?

Morse et al., Ann Surg 2013
Checkpoin blocka + BiTE


Courtesy: S. Patel
SW1463 cell line

1st round

2nd round

AsPC-1 cell line

1st round

2nd round

SW1463

Colo205

HT29

AsPC-1

no T cell

ContBITE/T cell

CEABiTE/T cell

CEABiTE/T cell 2nd Round
Fresh T cells

ContBiTE

Tumor alone

anti-PD1 + anti-PDL1

CEABiTE

annexin V

T cells after incubation with tumor cells/BiTE

ContBiTE/Tumor incubation

T cell from ContBiTE/Tumor incubation

anti-PD1 + anti-PDL1

CEABiTE/Tumor incubation

T cell from CEABiTE/Tumor incubation

IgG

anti-PD1

7AAD

annexin V
Anti-CTLA4 + antiPD-L1 + vaccine

Future strategies

- Later phase studies in resected adjuvant settings
- Combined vaccine + current group of immunomodulators
- Other immunomodulators (Anti-TIM3)
- Biomarkers of immune response
Collaborations

Surgical Sciences Applied Therapeutics/ Lyerly Lab
H. Kim Lyerly, MD
Takuya Osada, MD, PhD
Amy Hobeika, PhD
Zachary Hartman, PhD
Qing Cheng

Clinical/translational
Will Gwin, MD
Sandip Patel, MD
GI Onc/Phase I/Breast SBR
GU, GYN programs
Nelson Chao, MD/Stem Cell Txplnt

Gayathri Devi Lab
Amy Aldrich, Myron Evans

Neil Spector Lab

Smita Nair Lab

Industry
Alphavax Immunitope
Etubics Medimmune
Precision Biologics Aduro Newlinks

Wei Chen Lab/
Xiu-Ron Ren