Developing Tumor-Localized, Combination Immunotherapies by Arming the Oncolytic Group B Adenovirus Enadenotucirev

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“Armed” Enadenotucirev (EnAd): Tumour-Specific Immunogene Delivery Platform

1. EnAd is a chimeric group B oncolytic adenovirus currently being evaluated in phase I clinical studies for a range of carcinomas, including combination studies with chemotherapy & anti-PD1 antibody therapy
   • effectively delivered by systemic dosing to selectively replicate in tumour cells

2. Efficient and broadly applicable arming platform developed and exemplified

3. Produce functionally intact transgene-encoded proteins, while maintaining oncolytic properties of EnAd in vitro and in vivo

4. One, two and three distinct payloads exemplified

5. Payload expression using virus major late promoter restricts production to sites of virus replication in tumour cells – not detectable in non-tumour cells

Production of biologic therapeutics selectively within the tumour microenvironment
Enadenotucirev (EnAd):
Developed using directed evolution

- Start with a very large randomly created library of chimeric adenoviruses
- Passage the viruses repeatedly on human carcinoma cells
- Select only the most potent tumor killing viruses
- Screen candidate viruses for loss of activity in a variety of normal human cell types
- Select only the most selective and potent tumor killing viruses
- Screen candidate viruses on human carcinoma cells in the presence of fresh human blood
- Select the most potent, selective and blood stable tumor killing virus
A single IV dose of EnAd reduces tumour burden in A549 orthotopic lung cancer model

Untreated

25 days post EnAd treatment

>90% reduction in tumour burden

Single i.v. Dose of virus (5e9vp)
The key components of EnAd

EnAd = Ad11p with chimeric Ad3 E2B region and deletions in E3 and E4

Ad 11 capsid:
- Group B virus (vs C for Ad5)
- CD46 & DSG2 receptors
- No CAR receptor involvement
- Low pre-existing immunity against Ad11 surface proteins in humans

EnAd is human tumor-specific

Three deleted or mutated gene regions
- E3 and E4 deletions and a chimeric Ad3/Ad11p E2B region
  - E3 & E4: both regions normally involved in immune modulation by adenoviruses
  - E2B: adenovirus replication in the nucleus of normal cells
- Reduced genome size → capacity for arming
Armed virus particles are structurally the same as enadenotucirev

Encoded therapeutics expressed from virus major late promoter, products only made in cells supporting virus replication (i.e. tumor)
Arming EnAd to deliver immunotherapeutics to local tumour sites of action

Local immunotherapy delivery to tumours
- Effective concentrations in local microenvironments
- Minimized systemic exposure for improved safety
- Synergy with oncolytic virus properties

Supporting clinical data with enadenotucirev
1. Dose and dosing regimen established for systemic dosing
2. Acceptable safety/tolerability profile
   - Primarily acute reactions to particles or cytokines induced
   - No clinical evidence of virus replication
3. Virus is selectively detected in tumour cells and produces virus capsid protein
4. Evidence of T-cell response in tumours
Next Generation Viruses:

Tumor-specific immuno-gene therapy (T-SIGn)
### Panel of Research Viruses for Platform Evaluation & Exemplification:

#### Payloads & Viruses

#### Platform Development

<table>
<thead>
<tr>
<th>Payload</th>
<th>Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-VEGF</strong></td>
<td>NG-135 IgG1 Ab (IRES)</td>
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<tr>
<td></td>
<td>NG-165 IgG1 Ab (P2A)</td>
</tr>
<tr>
<td></td>
<td>NG-76, NG-73 ScFv Ab</td>
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<tr>
<td></td>
<td>NG-78, NG-74 ScFv Ab</td>
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<tr>
<td><strong>Reporters:</strong></td>
<td>NG-47, NG-62</td>
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<tr>
<td></td>
<td>NG-93</td>
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<tr>
<td></td>
<td>NG-105-109</td>
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<td>NG-159</td>
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<td>NG-61</td>
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<td></td>
<td>NG-63</td>
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<td>NG-282</td>
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#### Platform Evaluation

<table>
<thead>
<tr>
<th>Payload</th>
<th>Viruses</th>
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</thead>
<tbody>
<tr>
<td><strong>Anti-PD-L1</strong></td>
<td>NG-177 IgG1(m) Ab (IRES)</td>
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<tr>
<td></td>
<td>NG-190 IgG1 Ab (P2A)</td>
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<td></td>
<td>NG-221 ScFv Ab</td>
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<td><strong>Anti-CTLA-4</strong>:</td>
<td>NG-242 IgG1 Ab</td>
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<tr>
<td></td>
<td>NG-303 mIgG2a Ab</td>
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<tr>
<td><strong>TAA:</strong></td>
<td>NG-220</td>
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<tr>
<td></td>
<td>NY-ESO-1</td>
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<tr>
<td><strong>Cytokines:</strong></td>
<td>NG-139 TNFα</td>
</tr>
<tr>
<td></td>
<td>NG-95, NG-92 IFNγ</td>
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</tbody>
</table>
Properties of exemplifier antibody-armed EnAd NG-135 (full IgG\textsubscript{1} antibody)

**Virus Potency**

![Graph showing virus potency with EnAd and NG-135]

**Virus Replication in Carcinoma Cell Lines**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Genome copies per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td></td>
</tr>
<tr>
<td>HT29</td>
<td>10^5</td>
</tr>
<tr>
<td>DLD</td>
<td>10^4</td>
</tr>
<tr>
<td>HCT116</td>
<td>10^3</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td>A549</td>
<td>10^6</td>
</tr>
<tr>
<td>Ovary</td>
<td>10^7</td>
</tr>
<tr>
<td>SKOV</td>
<td>10^8</td>
</tr>
</tbody>
</table>

**Antibody expression**

![Image showing antibody expression with molecular weight markers]

**Anti-VEGF Antibody Expression**

![Graph showing anti-VEGF antibody expression over time for different cell lines]

Antibody ‘armed’ viruses efficiently replicate and express antibody in lung, colon and ovarian carcinoma cells
Functional activity of anti-PD-L1 antibody-producing viruses

MLR assays with human CD4:DC coculture (10:1) with neutralizing aPDL1 mAb at 2.5 \( \mu \)g/ml or 10x concentrated supernatants from aPDL1 (NG177 and NG190) or aVEGF (NG135 or NG165) armed viruses. IL-2 in supernatants at day 3

Antibody-containing supernatants from NG-177/NG-190 infected tumour cells can enhance human T cell activation (IL-2 production)
NG-135: Uptake, replication and antibody expression in tumour cells *in vitro*

**Antibody Production (72 hrs)**

<table>
<thead>
<tr>
<th></th>
<th>1ppc</th>
<th>10ppc</th>
<th>100ppc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-VEGF Ab (ng/ml)</td>
<td>1</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

**Infectivity**

HT-29 colon carcinoma cell line infected with NG-135 *in vitro*

**Anti-VEGF antibody production**

<table>
<thead>
<tr>
<th></th>
<th>1ppc</th>
<th>10ppc</th>
<th>100ppc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-VEGF Ab (ng/ml)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Virus replication**

<table>
<thead>
<tr>
<th></th>
<th>1ppc</th>
<th>10ppc</th>
<th>100ppc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus genomes/cell</td>
<td>10^3</td>
<td>10^4</td>
<td>10^5</td>
</tr>
</tbody>
</table>

**Virus replication is required for antibody production**
Lack of infectious virus or antibody production in stromal cells (non-transformed fibroblasts)

- Lack of detectable antibody: $< 0.33\text{fg/cell/24hr}$

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Viral genome copies per cell</th>
<th>Anti-VEGF Ab (ng/ml)</th>
<th>Infectious particles (TCID)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT29</td>
<td>$10^6$</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>WI38</td>
<td>$10^3$</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MRC5</td>
<td>$10^2$</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = not detected

- Lack of detectable antibody

- Over 2000-fold less antibody made by non-transformed cells than by cancer cells
EnAd and NG-135 replication and transgene production in primary hepatocytes

*Hepatocytes from peri-tumoural liver tissue

Genome replication and antibody expression: cells (3x10^5) infected with EnAd or NG-135 at 1ppc, cultured for 72hr

- Virus genome levels measured by qPCR
- Antibody expression measured by IgG1 ELISA

Replication & antibody production not detectable in human hepatocytes

ND = Not Detectable
Selectivity of Transgene Expression

Human Dendritic Cells

- NG-107: Virus Promoter-GFP
- NG-62: Virus Promoter-GFP
- NG-47: CMV Promoter-GFP

48h post co-culture with GFP-expressing EnAd

- No GFP expression in dendritic cells when virus promoter used
- Exogenous CMV promoter can drive gene expression (“transfection” via particle uptake)
Immunogene payload selection: How much and where needed?

1. Stay on or in the infected cell
2. Short range – released to act locally
3. Longer range – released and move to local lymphoid tissues
Antibody armed enadenotucirev (AbEnAd’s) = antibodies, antibody fragments or BiTe’s

NextGen virus

Tumor Cell

Full length Antibody A

Antibody Fragments A, B & C

BiTe D

Specific Ab binding and activity

Target A’

Target A’

Target B’

Target C’

Target D’

Anti-Tumor Immune Response
Tumour-secreted Immune Enhancers (T-sIE’s) = cytokines and/or chemokines

NextGen virus

Tumor Cell

Cytokine X

Cytokine X & Y

Cytokine X, Y & Z

Recruitment & Activation Signals

Anti-Tumor Immune Response
MiTe’s: Membrane-integrated T-cell engagers = T-cell activating ligands

NextGen virus

Tumor Cell

MiTe 1

Activation Signals

MiTe 2

Tumor Cell

MiTe 1

MiTe 2

Tumor Cell

T

T

T

Anti-Tumor Immune Response
CT-SIGN Therapies
Combinations to address multiple pathways

NextGen virus

Tumor Cell

MiTe 1

Ab Fragment A

Cytokine X & Y

Cytokine Z

Combined Recruitment & Activation Signals

Tumor Cell

Tumor Cell

Tumor Cell

MiTe 1

MiTe 2

Anti-Tumor Immune Response
Example NG combination viruses

NG Virus 1: MiTe1 + cytokine X
NG Virus 2: MiTe1 + cytokine X + Y

Effective expression of membrane and secreted proteins from the same virus

3 unique transgenes can be successfully secreted or inserted into the plasma membrane of treated tumor cells
NG combination virus 1: MiTe1 + cytokine X
Lack of expression of cytokine & MiTe by non-tumor cells
NG-348 is a first in class immune-gene therapy product for the treatment of carcinomas.

NG-348 forces *in situ* modification of the tumor cells such that they will be recognized by and activate any T-cell.

NG-348 is an off the shelf immunotherapeutic product for the treatment of solid tumors and is antigen-independent.

Contrast with CAR-T and TCR immunotherapies which modify T-cells via *ex vivo* manipulation with subsequent autologous transfusion, are antigen-dependent and show best promise in hematological malignancies.
NG-348: Lead T-SIGn Therapy

The Immunotherapy Challenge

Tumor Cell

No Activation

No Kill

Tumor-specific antigen

Normal T-Cell

The CAR-T / TCR Immunotherapy solution

T-cell is modified/expanded ex-vivo to express tumor-specific antigen receptors

T Cell

T Cell

Killing is independent of the tumor-specific antigen

The NG-348 T-SIGn Immunotherapy Solution

Tumor cell is modified in-situ to express T-cell activating ligands

Tumor Cell

NG-348 Activating Ligand

Killing is independent of the T-cell specificity

Kill

Activation via the antigen receptor

CAR-T-Cell

Kill

Killing is independent of the tumor-specific antigen
Approaches for evaluating immunomodulation by armed EnAd viruses

1. In vitro human DC/T-cell/Tumour cell co-culture systems
   - Innate & adaptive immune readouts

2. In vivo, immunocompetent mouse models
   - e.g. CT-26, B16 - using armed viruses with exogenous promoters (e.g. CMV)

3. In vivo human tumour xenografts
   - Immune competency via cell transfers, e.g. A549 lung model in SCID mice with immune cell transfers
1. EnAd is a potent & selective oncolytic virus, broadly active against epithelial cancer cells
2. Can be delivered to patients IV (clinically demonstrated) and can be efficiently armed with multiple therapeutic genes without disrupting oncolytic and tumor-selectivity properties of parental EnAd virus
   • Viruses encoding of variety of transgenes (e.g. antibodies, cytokines, tumor antigens) have been made and characterized
3. Excellent platform for tumor-specific immunogene therapy (T-SIGn)
   • Delivery and local production of immunotherapeutic combinations locally and selectively within tumours
   • Exemplified with multiple viruses expressing different classes and combinations of transgenes
4. NG-348, lead candidate ‡ selective expression of ligands on tumor cells to drive localized activation of T-cells