Arming the Oncolytic Virus Enadenotucirev to Develop Tumor-Localized Combination Immunotherapeutics

Charles Q. Morris MBChB MRCP(UK)
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

EnAd = Ad11/Ad3 group B chimeric adenovirus
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
Clinical PoC:
Tumor-selective expression of nuclear hexon staining after IV dosing

"Normal" Margin Tumor

Dark brown intra-nuclear staining of hexon demonstrates positive viral replication. Representative IV patient from the MoA study.
Clinical PoC:

Virus uptake into tumors is associated with CD8+ T-cell infiltrates

MoA CRC IV Cohort: 4/5 with high numbers of CD8+ cells amongst tumor cells (all CRC MSI low)
Next Generation Viruses: Tumor-Specific Immuno-Gene therapy (T-SIGN)
Armed Enadenotucirev to Deliver Immuno-Therapeutics to Local Tumor Sites of Action

Local immunotherapy delivery to tumors

- Effective concentrations in local microenvironments
- Minimized systemic exposure for improved safety

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 off-target virus replication
3. Virus is selectively detected in tumor cells and produces virus capsid protein
4. Evidence of T-cell response in tumors
Armed virus particles are structurally the same as enadenotucirev

Difference between armed and parental enadenotucirev

Encoded therapeutics expressed from virus major late promoter, products only made in cells supporting virus replication (i.e. tumor)
Properties of exemplifier antibody-armed EnAd NG-135 (full IgG₁ antibody)

Antibody ‘armed’ viruses efficiently replicate and express antibody in lung, colon and ovarian carcinoma cells.
NG-135: Uptake, replication and antibody expression in tumor cells in vitro

Antibody Production (72 hrs)

Anti-VEGF Ab (ng/ml)

Infectivity

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

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

\[ \text{Viral genome copies per cell} \]

- **HT29**: $10^6$
- **WI38**: $10^5$
- **MRC5**: $10^4$

\[ \text{Anti-VEGF Ab (ng/ml)} \]

- **HT29**: $10^2$
- **WI38**: ND
- **MRC5**: ND

\[ \text{Infectious particles (TCID}_{50} \]

- **HT29**: $10^8$
- **WI38**: ND
- **MRC5**: ND

**Cell Type**

**Replication**: Over 2000-fold less antibody made by non-transformed cells than by cancer cells

**Anti-VEGF Ab**: ND = not detected  \Rightarrow Lack of detectable antibody \Rightarrow < 0.33fg/cell/24hr

**Infectious Virus**: ND = not detected
EnAd and NG-135 replication and transgene production in primary hepatocytes*

* Hepatocytes from peri-tumoral 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
Next Generation Viruses

T-SIGn Examples
Cytokine Armed Enadenotucirev
cytokines and / or chemokines

NextGen virus

Tumor cell

Cytokine X

Cytokine X, Y

Cytokine X, Y, Z

Recruitment & activation signals

T cell

Anti-tumor immune response
NG-345 encodes three different cytokines/chemokines

- IFNα
- MIP-1α
- Flt3L

Cytokine production by NG-345 infected A549 lung carcinoma cells

- IFNα
- MIP-1α
- Flt3L

Three different human cytokines/chemokines produced by A549 tumor cells infected with a single T-SIgN virus
Membrane-integrated T-cell Engagers (MiTe): T-cell Activating Ligands

NextGen virus

Tumor cell

MiTe 1

MiTe 2

Activation signals

T cell

MiTe 1

MiTe 2

Anti-tumor immune response
NG-348 PsiOxus’ lead T-SIGN product

- NG-348 is a first in class immune-gene therapy product for the treatment of carcinomas.
- Forces membrane expression of T-cell activating ligands on tumor cells
- In situ activation of T-cells leading to antigen independent tumor cell killing
- Significant advantages over CAR-T and TCR technologies:
  - Off the shelf product: not personalized
  - Mass produced: no autologous manufacturing
  - Antigen independent: not dependent on CD19 or other antigens
  - Directed to solid tumors: not restricted to hematological malignancies
NG-348: Lead T-SIGN Immunotherapy

The Immunotherapy Challenge

Tumor cell

No activation

No kill

T cell

CAR T / TCR Immunotherapy

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

Kill

Activation via the antigen receptor

NG-348 T-SIGN Immunotherapy

Killing is independent of the T-cell specificity

NG-348

Killing

Activating ligand

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

Killing is independent of the tumor-specific antigen
NG-348
Selective expression of MiTe ligand on tumor cell surface

Only tumor cells infected with NG-348 express the MiTe
NG-348
Mediates T-cell expansion in the context of tumor cells

20 fold increase in T cell number post NG-348 stimulation

Starting with 10e6 T cells
NG-348

Infected A549 tumor cells
polyclonally activate human CD8+ T-cells

Infection and expression of NG-348 payload on human tumor cells leads to a direct and potent activation of CD8 cytotoxic effector T-cells.
NG-348
Selectivity of T cell activation: CD8 degranulation

No T-cell activation (CD8 effector function) induced by NG-348 treated non-transformed cells (MRC5 fibroblasts)
Antibody Armed Enadenotucirev
Antibodies, Antibody Fragments, or BiTes

NextGen virus

Tumor cell

Full length antibody A

Antibody (Ab) fragments A, B, C

BiTe

Specific Ab binding and activity

Target A’

Target A’

Target B’

Target C’

Anti-tumor immune response
Bi-specific T-cell Engagers (BiTEs) Targeting Cancer Associated Fibroblasts

Cancer Associated Fibroblast

- Sustaining proliferative signaling
- Evading growth suppressors
- Deregulating cellular energetics
- Avoiding immune destruction
- Resisting cell death
- Enabling replicative immortality
- Genome instability & mutation
- Inducing angiogenesis
- Activating invasion & metastasis
- Tumor-promoting inflammation

\( \alpha FAP \) antibody \( \Rightarrow \) BiTE \( \Rightarrow \) \( \alpha CD3 \) antibody

\( \Rightarrow \) Fibroblast-targeted T-cell activation
Bi-specific T-cell Engagers (BiTEs)

Infection with EnAd-FAP-BiTE induce T-cell activation

Activation

\[
\text{% CD69}^+ \\
\text{% CD25}^+ \\
\text{Time (h)}
\]
Bi-specific T-cell Engagers (BiTEs)

Infection with EnAd-FAP-BiTE kills stromal fibroblasts

EnAd-FAP-BiTE induces rapid killing of stromal fibroblasts
Bi-specific T-cell Engagers (BiTEs)
Ovarian malignant ascites as an ex-vivo human model

- Rich content of tumour-associated lymphocytes, fibroblasts, tumour cells, macrophage

- Immunosuppressive T-cells are:
  - PD1-positive
  - Attenuated activation markers + proliferation of T-cells
  - IL-2 non-producers
Bi-specific T-cell Engagers (BiTEs)
Ovarian Malignant Ascites “treated” with EnAd-FAP-BiTE

EnAd-FAP-BiTE treatment of unseparated ovarian cancer ascites cells leads to activation of T-cells and depletion of FAP+ cells.
Combination Therapies
Combinations to Address Multiple Pathways

NextGen virus

Tumor cell

MiTe 1
Ab fragment A

Combined recruitment & activation signals

MiTe 1
Cytokine X, Y

MiTe 1
MiTe 2
Cytokine Z

T cell

Anti-tumor immune response
# Pipeline
## R&D programs and progress

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Summary

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**
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