Developing tumor-localized, combination immunotherapies

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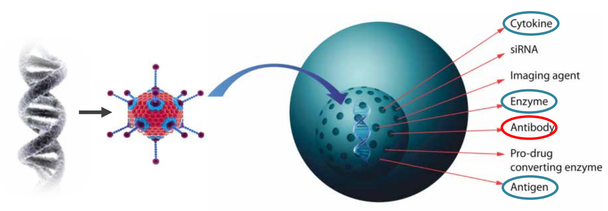
"Armed" EnAd Platform Overview

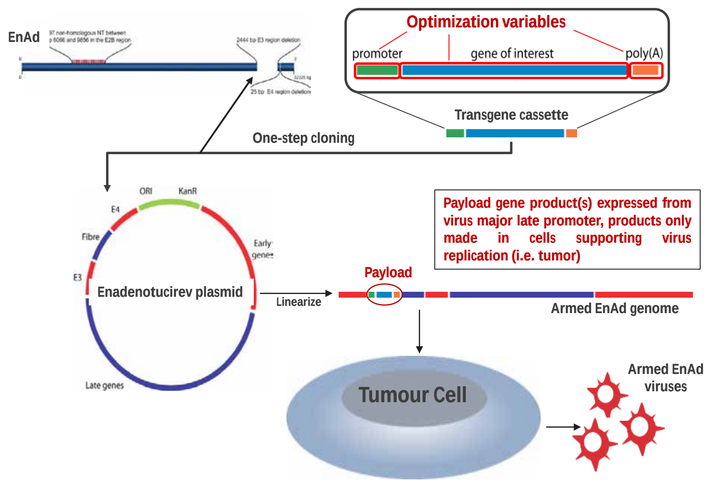
Tumour-specific delivery of immunotherapy combinations

1. Enadenotucirev (EnAd) is an Ad11p/Ad3 chimeric group B oncolytic adenovirus that uses CD46 and DSG2 receptors to infect cells, unlike Ad5 (a group C virus) which uses the CAR receptor
2. Clinical studies have shown that EnAd can be dosed systemically to cancer patients and is effectively delivered to and selectively replicates in tumor cells
3. The chimeric E2B region and E3/E4 region deletions (~2.5kb) in EnAd drive the high potency and human tumor cell selectivity of this virus, and provide "space" for encoding transgenes
4. An efficient and broadly applicable EnAd arming platform has been developed and exemplified with a variety of biotherapeutic agents: antibodies, cytokines etc. Viruses with one, two and three distinct payloads have been exemplified
5. The platform produces armed viruses that produce functionally intact transgene-encoded proteins, while maintaining oncolytic properties of EnAd *in vitro* and *in vivo*
6. Payload expression using virus major late promoter restricts production to sites of virus replication in tumor cells – not detectable in non-tumor cells



Production of biologic therapeutics selectively within the tumour microenvironment

Armed EnAd Virus Platform



Platform Development

Payload	Viruses
Anti-VEGF :	NG-135 IgG1 Ab (IRES) NG-165 IgG1 Ab (P2A) NG-76, NG-73 ScFv Ab NG-78, NG-74 ScFv Ab
Reporters: (GFP or luciferase)	NG-47, NG-62 NG-93 NG-105-109 NG-159 NG-61 NG-63 NG-282

Platform Evaluation

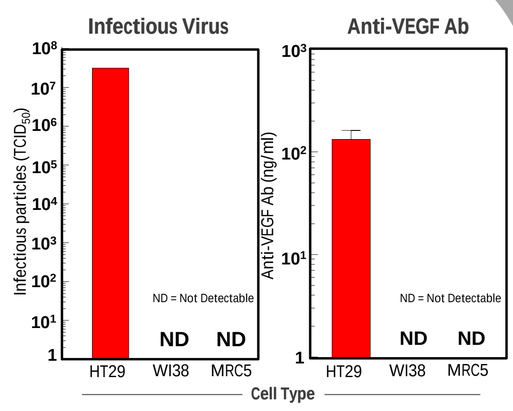
Payload	Viruses
Anti-PD-L1 :	NG-177 IgG1(m) Ab (IRES) NG-190 IgG1 Ab (P2A) NG-221 ScFv Ab
Anti-CTLA-4:	NG-242 IgG1 Ab NG-303 mIgG2a Ab
TAA:	NG-220 NY-ESO-1 NG-217
Cytokines:	NG-139 TNFα NG-95, NG-92 IFNγ

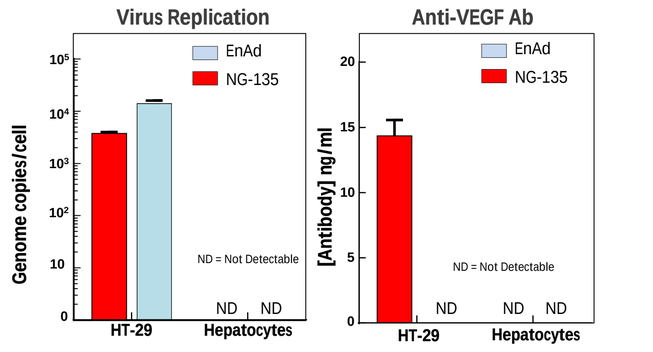
Selectivity of Armed EnAd Viruses

Example Armed EnAd

NG-135 = EnAd armed with anti-VEGF antibody H&L chains

- à produces fully functional antibody without affecting oncolytic properties of EnAd
- à Used to evaluate tumor-selectivity of both virus replication and payload production

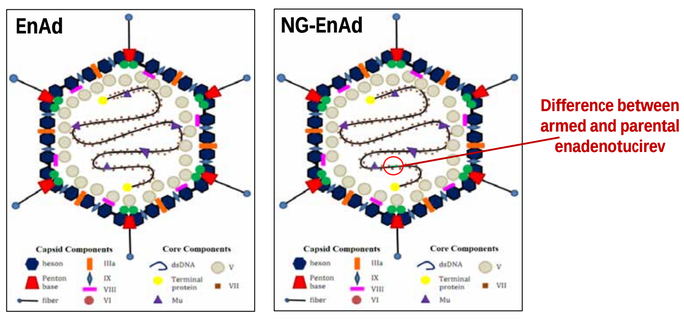




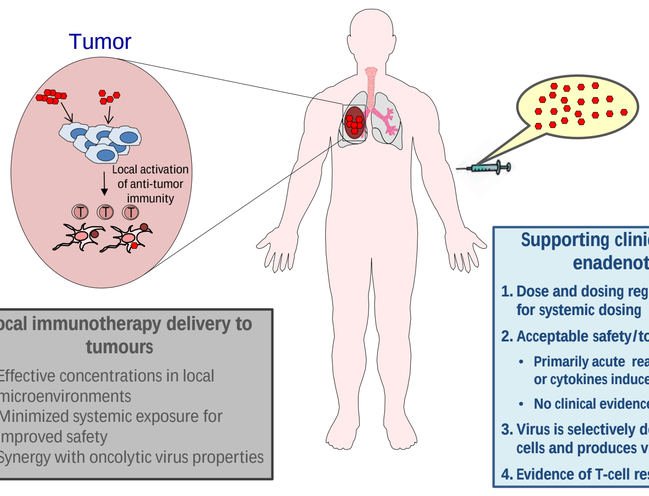
ND = not detected à Lack of detectable antibody à < 0.33fg/cell/24hr

à Over 2000-fold less antibody made by non-transformed cells than by tumor cells

Armed virus particles are structurally the same as enadenotucirev



Arming EnAd to deliver combination immunotherapeutics to local tumor sites of action



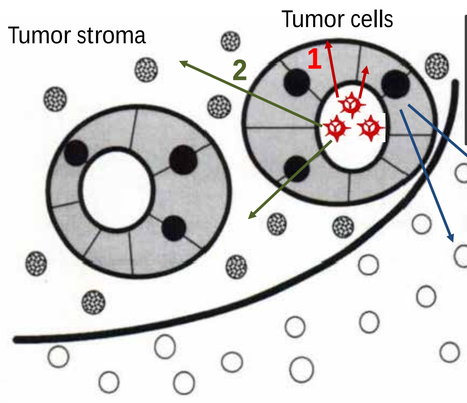
Local immunotherapy delivery to tumours

- 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 tumor cells and produces virus capsid protein
4. Evidence of T-cell response in tumors

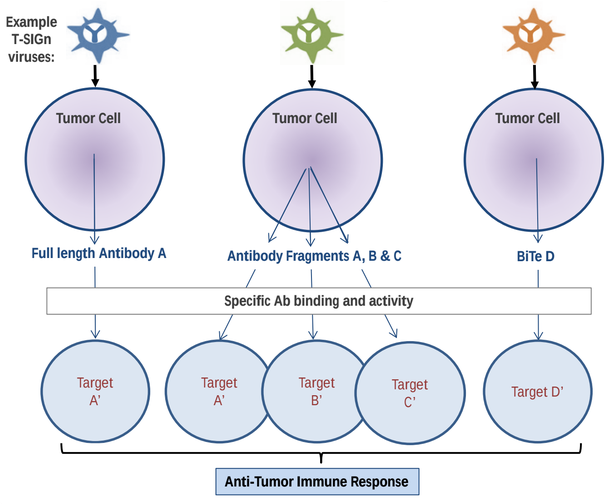
Immunogene payloads can be selected to generate a combination of products that modulate the tumor microenvironment in different ways



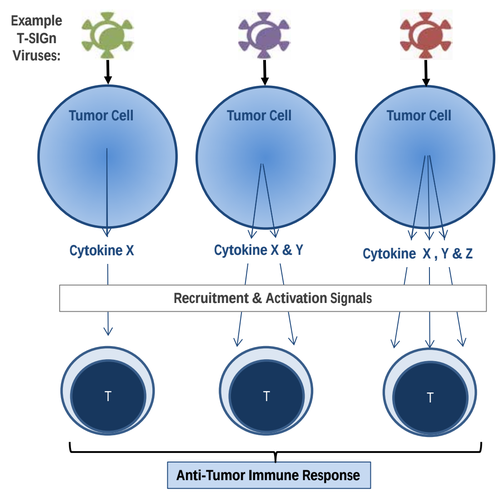
1. Stay on or in the infected cell
2. Short range – released to act locally
3. Longer range – released and move to local lymphoid tissues

à Candidate Next Generation Viruses à Tumor-Specific Immuno-Gene therapy (T-SIGn)

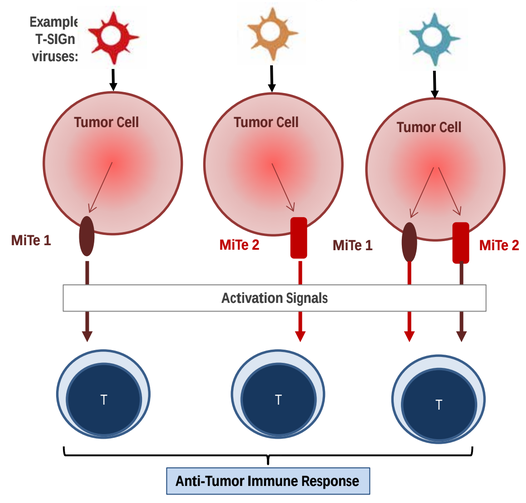
Antibody armed enadenotucirev (AbEnAd's)
= antibodies, antibody fragments or BiTe's



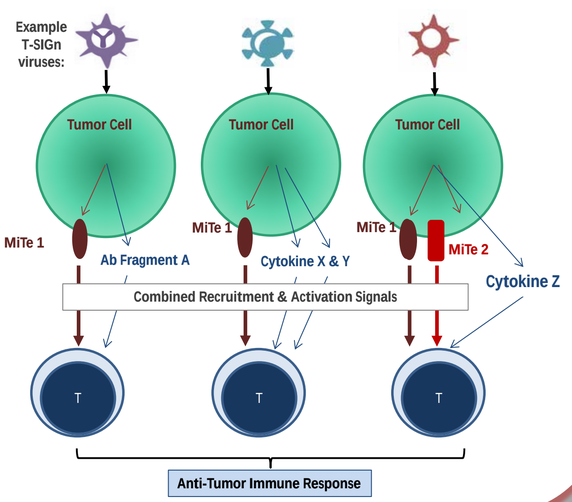
Tumour-secreted Immune Enhancers (T-sIE's)
= cytokines and/or chemokines



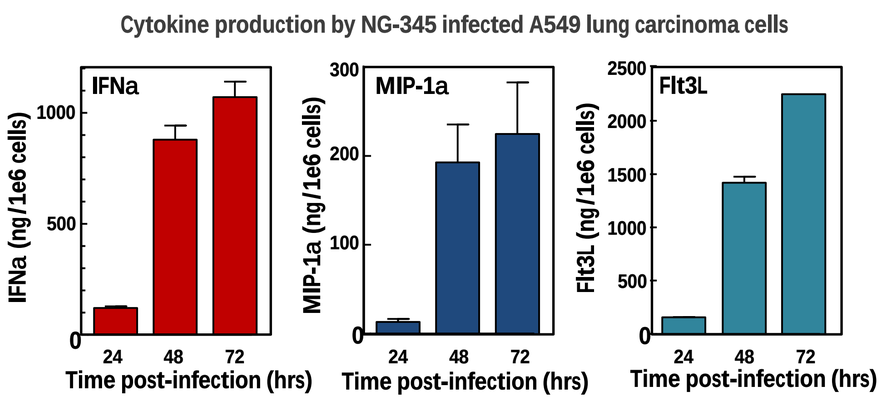
MiTe's: Membrane-integrated T-cell engagers
= T-cell activating ligands



CT-SIGn Therapies
Combinations to address multiple pathways

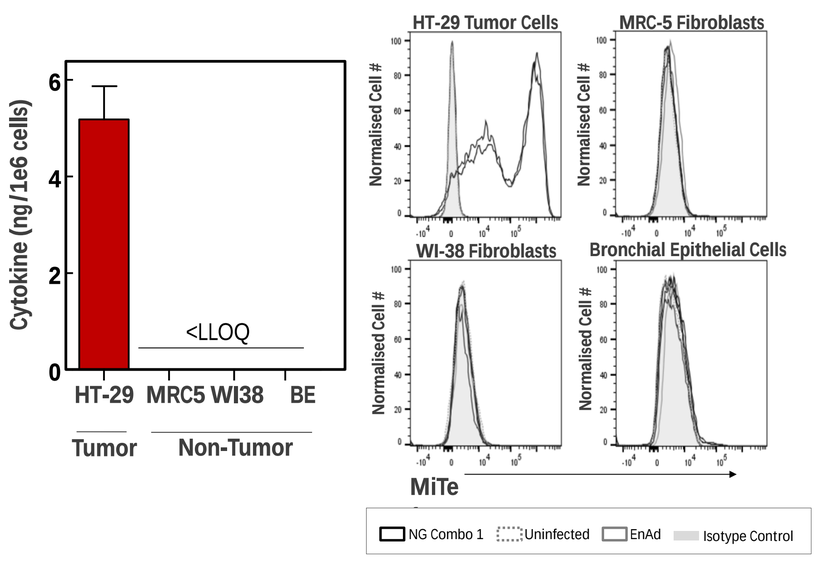


NG-345: Cytokine/Chemokine Combination Virus

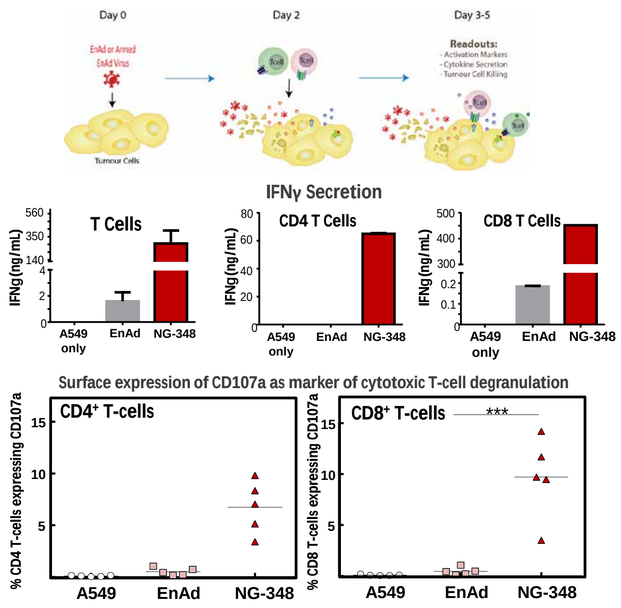


Ø Three different human cytokines/chemokines produced by A549 tumor cells infected with NG-345, a single NG virus encoding three separate transgenes

NG combination virus (NG-343): MiTe1 + cytokine
Lack of expression of cytokine & MiTe transgenes by non-tumor cells



MiTe virus (NG-348) infected A549 cells potently activate human T-cell effector functions



Summary

We have developed a broadly applicable vector platform system, based on the potent chimeric oncolytic group B adenovirus enadenotucirev (EnAd), for directing the efficient local production of a combination of immunotherapeutic agents selectively within the tumor. The versatility and fidelity of the platform has been exemplified by encoding up to three separate biomolecules in the same virus, including antibodies, cytokines, chemokines and membrane integrated T-cell activating ligands, without altering other virus properties. A systemic clinical dosing regimen has been established for EnAd, with data directly demonstrating selective virus delivery to and protein production from colorectal and other tumor types. The advantage of this approach is that immunotherapeutics encoded in the virus can be produced locally, both in tumors that are not directly injectable and in metastases, while minimising systemic off-target effects.