The CMC challenges in Developing an Oncolytic Immunotherapy

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Vaccine Technology IV
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Provided May 22 2012, as part of an oral presentation and is qualified by such, contains forward-looking statements, actual results may vary materially; Amgen disclaims any duty to update.
Talimogene laherparepvec (formerly OncoVEX<sup>GM-CSF</sup>)

- talimogene laherparepvec

- Name Derivation:
  TA limo gene LA herpa rep vec
  - immuno-modulating
  - gene therapy
  - herpes simplex virus based
  - replicating
  - vector

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BioVex

- Boston based company focused on biologics for cancer and infectious disease
  - Founded in 1999 at UCL; Opened US site in 2005
  - Acquired by Amgen March 2011
- Lead product talimogene laherparepvec (formerly OncoVEX\textsuperscript{GM-CSF})
  - Activity demonstrated in multiple tumor types
  - Phase 3 melanoma study commenced in 2009
  - Recruitment completed July 2011
- ImmunoVEX\textsuperscript{HSV2}
  - Live attenuated vaccine for genital herpes
  - Dosing in Phase 1 trial commenced in 2010

Liu et al., Gene Therapy 2003
CMC challenges of an Oncolytic Immunotherapy

- How does talimogene laherparepvec work?
- Does talimogene laherparepvec work?
- Bio-safety challenges in manufacturing a live viral product
  - Pre-clinical safety challenges with a viral product
  - Manufacturing bio-safety
  - Assuring viral safety of the product
- Manufacturing challenges of live viral production
- Analytical challenges of a product with a dual mode of action
Innovative Mechanism of Action

**Talimogene Laherparepvec**
Investigational Oncolytic Immunotherapy

Talimogene laherparepvec is an oncolytic herpes simplex virus type 1 engineered to replicate selectively in tumor cells and to express granulocyte-macrophage colony-stimulating factor. Upon injection into accessible tumors, the proposed mode of action includes:

- Necrosis of injected tumors due to virus replication
- Induction of immune response against tumor antigens, leading to an effect on uninjected lesions

**LOCAL EFFECT**

1. **Talimogene laherparepvec** is an attenuated virus that invades both tumor cells and normal healthy cells.
2. **Talimogene laherparepvec** selectively replicates and generates GM-CSF in tumor cells.
3. Tumor cells rupture to release replicated viruses and GM-CSF; TSAs are exposed.

**SYSTEMIC EFFECT**

1. Replicated viruses repeat cell lysis cycle in nearby tumor cells.
2. GM-CSF recruits dendritic cells to tumor sites.
3. Dendritic cells process and present TSAs to mediate a tumor-specific immune response.
4. Adapts immune response identifies and destroys tumor cells systemically.

The above depiction is believed to be the mechanism of action of talimogene laherparepvec. This compound is investigational.

**REFERENCES:**
Evidence of Destruction of Un-Injected Tumors

Mouse A20 lymphoma, tumors in both flanks

Liu et al., Gene Therapy 2003
Evidence of Tumor Necrosis

Hu et al., Clin Cancer Res 2006
Replicates in the Tumor

PFU / swab

1000

800

600

400

200

0

3d 4d 5d 6d 7d 8d 9d 10d

Days post dose of $10^7$ PFU/mL on day 0

Hu et al., Clin Cancer Res 2006
Evidence of Tumor Necrosis

melanoma, three injections : $10^6, 10^8, 10^8$ pfu/ml

BioVex and Amgen data on file
Evidence of Tumor Selectivity

- Patient biopsies showed necrosis, all with strong HSV staining
- Unlike studies with previous oncolytic viruses, staining was spread throughout the tumor
- HSV staining was only rarely seen in non-necrotic tumor tissue and even more rarely in non-tumor tissue

Hu et al., Clin Cancer Res 2006
Local Production of GM-CSF in Tumor

relative mRNA levels at 48 hours

Hu et al., Clin Cancer Res 2006
Example of Results Observed from Melanoma Trial

Baseline 6 months 9 months

BioVex and Amgen data on file
Example of Results Observed in Melanoma Trial: Uninjected Sites

Senzer et al. JCO 2009;27:5763-5771

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Right Shoulder – Baseline and 6 months

*No other lesion was ever injected in this patient

Senzer et al. JCO 2009;27:5763-5771
Overall Survival

12-month OS:
- 58% (all pts and all stage IV)
- 40% (M1c)
- 93% (responders)

Senzer et al. JCO 2009;27:5763-5771

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Safety challenges in manufacturing a live viral product

- Pre-clinical safety challenges with a viral product
- Manufacturing bio-safety
- Assuring viral safety of the product
CMC issues in manufacturing a live virus product

- Design and Construction of a safe virus
- Pre-clinical safety challenges with a viral product
- Manufacturing bio-safety
- Assuring viral safety of your product
At which point in the project do you focus on Bio-safety?

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Why select Herpes simplex virus ‘HSV?’

- Causes cold sores, genital herpes
- Same family as VZV, EBV, CMV
- Extremely well characterised
- Pathology well understood
- Can replicate in most dividing cells
- Large non-integrating DS DNA genome
- Possible to accommodate large genetic inserts
- Pre-existing immune response does not block reimmunisation
- Replication halted by acyclovir
- Known mutations supply tumour-specific growth
Construction of a safe and effective Oncolytic virus

- Tumor selective replication
  - Delete ICP 34.5
- Enhance replication in tumors
  - Engineer clinical isolates of HSV
  - Increase US11 expression
- Increase systemic immune effects
  - Delete ICP47 to prevent block of tumor cell antigen presentation
  - Incorporate GM-CSF
Preclinical safety challenges

- Selection of pre-clinical efficacy models – challenges of a human virus
- GLP toxicology - selection of suitable animal model and strain
  - use in combination studies (chemo-radiation)
  - use to support process changes
  - Murine vs human GM-CSF
- Biodistribution - requires high sensitivity & specificity from qPCR assay
  - special tissue handling methods
  - key to designing clinical protocol and human distribution studies
- Potential of latency and virus reactivitation
  - poor laboratory models
- Cell bank and virus seed stock testing
- Virus characterisation and sequencing
- Proof of concept for viral safety features
  - tumour specificity
  - GM-CSF production and distribution
  - virus shedding
CMC challenges in manufacturing a live viral product

- Manufacturing challenges of live viral production
- Analytical challenges of a product with a dual mode of action
- Regulatory considerations for an oncolytic virus
Manufacturing Bio-safety

- **Design of Manufacturing facility**
  - Early development: Multi use CMO & filling facilities
  - Later development & commercial: custom design single use facility
  - Use pressure cascade to contain and protect product

- **Process design**
  - Low pressure to minimise spills and aerosols
  - Risks increase with increasing volume
  - Minimise shear forces
  - Maximise disposable use
  - Terminal filtration vs asceptic processing

- **Operator safety and training**

- **Additional cleaning validation challenges**
Manufacturing process

Cell Build

Harvest

Benzonase Treatment

Filter Clarification

Tangential Flow Filtration

2 Chromatography steps

Sterile Filtration/Filling

4 Weeks

1 Week
Viral safety when your product is a live virus

• Follow standard ICH/Ph.Eur/FDA guidelines
• Raw material testing
• Cell bank and Viral bank testing (MCB, WCB, MVSS, WVSS)
• Clinical batch testing at virus harvest stage testing vs protection of product
  * in vitro adventitious testing on reporter cell lines*
  * in vivo adventitious testing*
  * retroviral testing*
  * impossible without good blocking antisera and dilution
• Mycoplasma testing*
Analytical challenges of a dual mode of action

Release Testing
- Demonstrate consistency and quality of product

Characterisation
- Define product and manage change

Clinical Trial Support
- Bio-distribution
- Virus containment
- Efficacy evidence

Stability Testing
- Demonstrate product stability and determine shelf life

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Herpes Simplex Virus (HSV)

- large, double stranded DNA virus (150Kb genome)
- virus particle diameter 180 – 200 nm
- encodes for almost 80 proteins
- virus particles composed of 4 structural elements

Modified from Granzow et al., 1997
Batch release testing for Immunotherapy - the challenges

- Immunotherapy products may have more than one mode of action
  - Potency Assay(s)
    - Infectivity (Plaque assay)
    - GM-CSF assay of biological activity
  - Purity
    - Intact Particle (SEC)
    - Dissociated Proteins (Coomassie gel/ HSV Western Blot)
  - Impurities
    - BSA, Benzonase®
    - Inactive HSV, host cell DNA
    - Host cell protein
  - Content
    - Total Protein (Protein Assay)
    - Viral Protein (HSV-1 ELISA)
  - Identity
    - Southern Blot
  - Safety

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Product characterisation - How do you manage process change?

- Can you demonstrate comparability for immunotherapeutics?

- Animal preclinical studies - safety
  - efficacy

- In vitro growth curves

- Tumour cell killing

- Potency bioassay

- Physical characterisation (SEC, light scattering, density gradients, EM)

- Protein characterisation (SDS Page, MAb binding, western blots, sequencing, ELISA)

- DNA characterisation (sequence, fingerprint, qPCR)

- Comparison of impurities

- Accelerated stability studies (elevated temperatures & freeze thaw cycles)
A20 Tumour Model

- Clears Mouse A20 lymphoma, in both injected and uninjected tumours
Characterisation – Particle Size Analysis

Zetasizer Analysis – Particle Size

Particle Size Distribution Curves

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Dissociated Protein Quantitation

2D PAGE
- Clean images with distinct spots
- Spot pattern visually similar between batches
- Quantitative analysis

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CMC Challenges of a Oncolytic Immunotherapy

Conclusions

- Increased expectation of purity & safety
- Increased expectation of consistency
- Complexity of characterisation
- Potency determination
- Product robustness
- Product containment
- Small scale production

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