EMEA-ICH Workshop on Viral/Vector Shedding Symposium

Case Study: Ad2/HIF-1a/VP16: Experience with Viral Shedding & Circulation Testing

Adenoviral Mediated Angiogenic Gene Therapy Study for Patients with Intermittent Claudication (WALK Study)

US, UK and GER

30 Oct 07

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WALK

A CLINICAL RESEARCH STUDY ON INTERMITTENT CLAUDICATION

A Phase II Study of Ad2/HIF-1α/VP16 in Patients with Intermittent Claudication

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Phase 2 Study Design

- Assess the safety and efficacy of HIF-1α in the treatment of patients with intermittent claudication (IC)
- Randomized, double-blind, placebo-controlled (RDBPC) parallel arm* dose selection study
- 3 doses across a 2 log dosing range
  - 2 x10^{11} vp, 2 x10^{10} vp, 2 x10^{9} vp
  - Placebo (PBS + 10% sucrose)
- IM administration to both limbs using a grid to standardize placement of injections (20 100μL IM injections to each limb)
- 75 patients per group, for a trial size of 300 patients

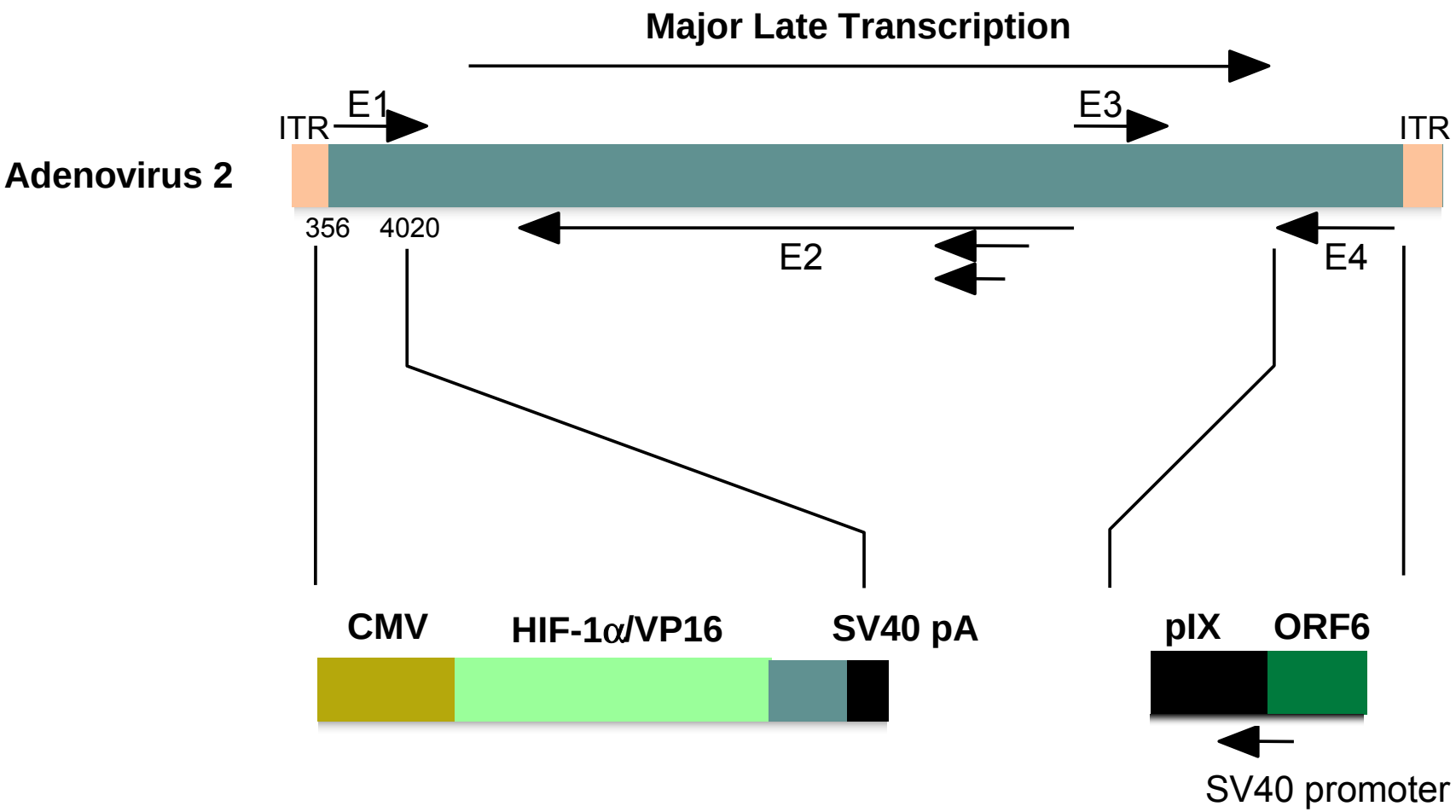
*TASC Management of PAD, J Vasc Surg 31:1, 2000;
EMEA/CPMP Guidance on Clinical Investigations in PAOD, 2002

Study Endpoints

- Primary Efficacy Endpoint:
  - Change from baseline in **Peak Walking time (PWT)** compared to placebo at 6 months*
- Safety Endpoints (Out to 2 years followed by EFUP)
  - Adverse events
    - Focus on potential risks of transgene and it's delivery system
      - Injection site reactions
      - Adenovirus-related infections
      - Pathological neovascularization (proliferative retinopathy, new malignancies)
      - Major adverse vascular events and related hospitalizations
  - Antibody titers and neutralizing antibody titers
  - Monitoring for viral shedding

*TASC Management of PAD, J Vasc Surg 31:1, 2000;
EMEA/CPMP Guidance on Clinical Investigations in PAOD, 2002

Ad2/HIF-1α/VP16 Vector



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Background on Testing Adenoviral Vector Shedding

- Following IM administration of a replication-deficient adenoviral vector, the vector must cross multiple biologic barriers to reach an epithelial surface and be shed.
- These barriers include multiple tissues and the host's immunity.
- It is much more likely that the vector will infect a cell, or be neutralized by the immune system during its transit period.
- Adenoviral shedding not observed after IM, IMC or IC delivery in previous adenoviral mediated angiogenesis studies* (2004)
- Also not observed in previous GEN studies with same vector viral backbone:
  - Nasal, intra-lobular or aerosol administration in CF patients

*Joseph, 2001, *Hum Gene Ther;* Grines, 2002, *Circulation;* Rajagopalan, 2003, *Circulation*

Rationale for Testing Adenoviral Vector Shedding

- However, at that time studies often only evaluated a subset of the entire study patient population
- US, UK and GER regulatory and competent authorities suggested:
  - Conduct of a rigorous assessment in WALK Study with viral shedding results to be shared with regulators
  - These data would be very useful for guidance in future adenoviral mediated gene therapy clinical studies
- As a sponsor, GEN would like to stop this very labor intensive testing if appropriate (i.e., viral shedding not a safety issue) in future studies

Plan for Vector Shedding & Circulation Testing

- Designed as appropriate for IM route administration
  - Local delivery into skeletal muscle
- Selection of specimens based on characteristics of preclinical viral distribution studies and adenovirus metabolism
  - Throat swab (respiratory) and urine (renal)
  - Fecal testing (hepatic) not possible (difficulty with assaying)
  - Selection of specimen for remote possibility of shedding in semen (just to be sure although non-integrating vector)
- Selection of timepoints for vector testing based on preclinical vector biodistribution studies
  - One, three and seven days
- GEN performed vector circulation; internal decision
  - Clearance pattern from blood would determine window for potential shedding risk

Extent of Testing

- Determined by guidance from US, UK and GER:
  - Regulatory agencies
  - Gene therapy competent authorities
- Study data monitoring committee (DMC) guidance
  - Collect specimens on all 300 study patients
  - Perform “real time” analysis on first 60 patients
  - DMC review to determine if additional “real time” analysis
  - Maintain frozen samples if further testing warranted
- Keep remaining specimens if further testing warranted
  - Need ongoing specimen stability studies if further testing conducted

Viral Shedding and Circulations Assays

Adenoviral Shedding

- Specimens analyzed at Genzyme Clinical Immunology Laboratory.
- An adenoviral-shedding cell-based adenoviral cultivation assay includes an assessment of adenoviral infectivity in HEK293 cells permissive for both recombinant and wild type adenovirus.
- If an adenovirus is detected, it will be tested in the vector-specific PCR assay to determine if it is the Ad2/HIF-1α/VP16 vector.

Vector Circulation

- The vector-specific PCR assay is used to determine if there is Ad2/HIF-1α/VP16 vector circulating in the blood.

More SOM Directions the Better, Dry Runs Invaluable (1)

- Clear reference to when specimens should be collected
  - Simple checklist helps as a quick reference
  - Laminated card with color coded listings; what/when /how
- Detailed description of required collection, processing, packaging and shipping procedures
  - Check box to track after each step is completed
  - Devils in the details more inclusive the better
    - Training video showing how to use the various lab kits
    - Dry runs can be very helpful, continue to refine processes
  - Include specific references to prevent easily forgotten details which could compromise specimens
    - “Do Not Thaw”,
    - “Use Dry Ice” (and make sure site has a source),
    - “Use cold pack provided”

More SOM Directions the Better, Dry Runs Invaluable (2)

- Provide a cheat sheet which clearly displays under what conditions each specimen must be shipped
- Validate shipping containers to maintain required storage conditions for the duration of the shipment process (could be different for different regions)
- Provide clear instructions for days of the week when samples can and can not be shipped based on delivery timeline (e.g., not Friday)
- Use tried and true shipping company
- Recommend batching of samples:
  - Will depend on clinical laboratory testing timelines
  - Also site storage capacity

More SOM Directions the Better, Dry Runs Invaluable(4)

- Site must have alarms and back up power source for freezers
- Temperature tracking log required to document proper conditions
- Stay in the loop with the Clinical Specialty laboratory to receive feedback on their experience with receiving samples
  - Corrective action may be required for a site and should be addressed as early as possible

Assay Development to Clinical Specialty Laboratory

- Research laboratory not adequate for adequate assay validation and processing under controlled systems except for initial small trials
- Research laboratory will develop research assay:
- Don’t underestimate difficulty in assay development with certain types of specimens (sputum, urine and especially semen) can be quite challenging
- Research assay must be transferred to clinical laboratory for validation and scale up
- Clinical specialty laboratory with dedicated resources required for analyzing multiple samples
- Need to establish data transfer process which can be converted to SAS ready data sets for clinical database