# A Randomized, Double-blind, Placebo-controlled, Parallel-group, Study of Ad2/Hypoxia Inducible Factor (HIF)-1$\alpha$/VP16 in Patients with Intermittent Claudication

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NIH/OBA Protocol # 0407-661

# Walk Study Steering Committee

| | |
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# Investigators and Study Sites

35 sites: United States 27; United Kingdom 4; Germany 4

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- William Marston – UNC Chapel Hill
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# Background and Rationale

- Peripheral artery disease affects 8-10 million persons in the US, of whom approximately 30 percent have symptoms of intermittent claudication.
- There are few medical therapies for claudication, and revascularization is usually reserved for those with disabling symptoms or critical limb ischemia.
- Therapeutic angiogenesis via gene therapy has the potential to ameliorate claudication by forming new blood vessels and improving blood flow in affected limbs of patients with PAD.

# HIF-1α
## The Master Switch for Hypoxic Stress

"The Cellular Oxygen Sensor"
Induces the Activation of

↓

**HIF-1α**

**Cell Survival**
**Glycolytic Enzymes**
**iNOS**
**IGF-2**
**IGF-Binding Proteins 2+3**
**TGF-beta3**
**BNP**

**Angiogenesis**
**VEGF-A (all isoforms)**
**VEGF-R1**
**PlGF, PDGF**
**PAI-1, SDF-1**
**Angiopoietins**
**Mediators of Inflammation and arteriogenesis**

**Vascular Tone**
**iNOS**
**eNOS**
**HO-1**
**$\alpha_{1B}$-Adrenergic Receptor**

**Iron/Oxygen Metabolism**
**Erythropoietin**
**HO-1**
**Transferrin**
**TfR**
**Ceruloplasmin**

# Background and Rationale

- The HIF-1$\alpha$/VP16 hybrid transcription factor is comprised of a truncated HIF 1$\alpha$ sequence fused to a herpes simplex virus (HSV) VP16 promoter.
- HIF-1$\alpha$/VP16 increases collateral blood vessel formation and blood flow in experimental models of hindlimb ischemia.
- In a phase 1 trial of patients with critical limb ischemia, HIF-1$\alpha$/VP16 was safe and associated with resolution of rest pain and ulcer healing in some patients.

# Hypothesis

- Intramuscular administration of Ad2/HIF-1$\alpha$/VP16, an engineered recombinant type 2 adenovirus vector encoding constitutively active HIF-1$\alpha$, will improve peak walking time in patients with PAD and intermittent claudication

# Objectives

- To assess the efficacy of HIF-1$\alpha$/VP16 compared to placebo in the treatment of severe intermittent claudication
- To assess the safety and tolerability of HIF-1$\alpha$/VP16 compared to placebo in patients with severe intermittent claudication

# Inclusion Criteria

- PAD affecting both legs
- Stable claudication for at least 6 months
- ABI of the index (most symptomatic) limb ≤ 0.90 at rest (or TBI ≤ 0.70).
- ABI must decrease ≥ 20% after exercise in both legs
- ≤25% difference in PWT between two sequential baseline exercise tolerance tests (ETTs) at least 1 week apart
- PWT between 1 and 12 minutes

# Exclusion Criteria

- Critical limb ischemia
- Aortoiliac disease that limits blood flow to the index limb
- Peripheral surgical revascularization procedure within 6 months or endovascular revascularization within 3 months of enrollment
- Planned peripheral revascularization procedure within 6 months.
- Nonatherosclerotic PAD (e.g. Buerger's disease)
- Walking impairment from other causes (e.g. arthritis, neuropathy)
- Conditions that limit walking (e.g. angina, CHF, COPD)
- Cancer within 5 years

# Cancer Screening

- American Cancer Screening Criteria applied to all patients (including EU)
  - Chest X-ray
  - Colonoscopy,flexible sigmoidoscopy,or double contrast BE-screening
  - Stool for occult blood
  - PSA
  - Mammogram
  - Pap and Pelvic Exam
- Any abnormal finding required specialist follow up

# Additional DMC Requirements

- Key laboratory parameters and non-specific but potential early malignancy findings at screening must be discussed with sponsor and adequately documented:
  - ESR ≥ 50 mm/hr
  - PSA > 4 ug/L
  - Lab parameters ≥ CTCAE v.3.0 Grade 3
  - Urine RBC ≥ 2+ Pulmonary nodules
  - Other potential early malignancy findings

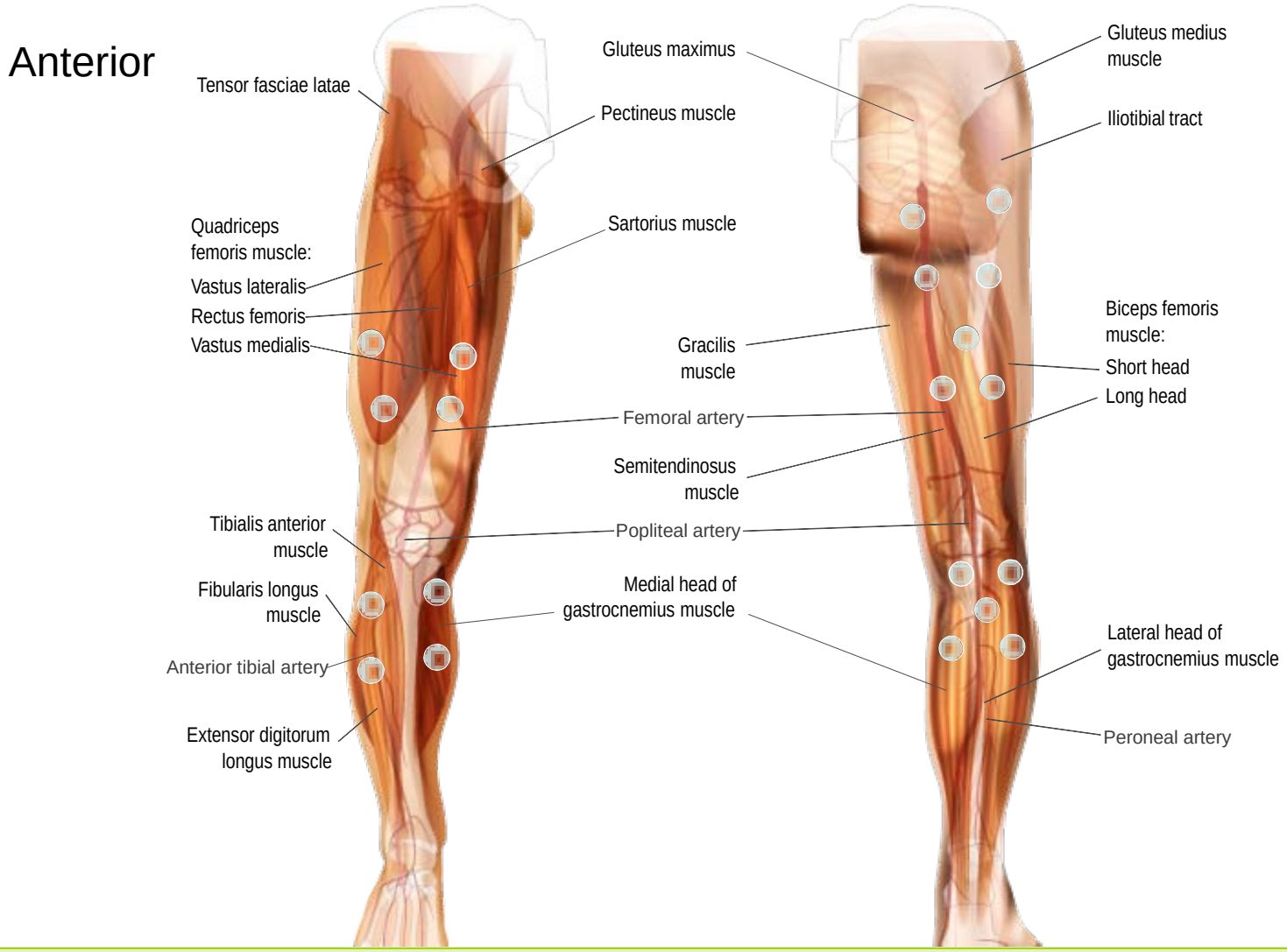
# Experimental Protocol

- Patients were randomly assigned to receive a single treatment with 1 of 3 doses of Ad2/HIF-1α/VP16, or placebo, in a ratio of 1:1:1:1, in a double blind manner.

| Dosing Group | Dose per Limb | Injections per Limb |
|---|---|---|
| Ad2/HIF 1α/VP16 $2x10^9$ vp | $1x10^9$ vp | 20 |
| Ad2/HIF 1α/VP16 $2x10^{10}$ vp | $1x10^{10}$ vp | 20 |
| Ad2/HIF 1α/VP16 $2x10^{11}$ vp | $1x10^{11}$ vp | 20 |
| Placebo | NA | 20 |

- Each injection consisted of a fixed volume of 100 μL for a total volume of 2.0 mL per limb.

# Treatment Administration
## Standardized Grid for Injections



# Efficacy Endpoints

- Primary Endpoint:
  - Percent change from baseline in **Peak Walking Time** at 6 months
- Secondary Endpoints:
  - Percent change from baseline in **Peak Walking Time** at 3 and 12 months
  - Percent change from baseline in **Claudication Onset Time** at 3, 6, and 12 months
- Exploratory Endpoints
  - Endothelial progenitor cells
  - Vascular endothelial growth factor (VEGF)
  - Adenovirus antibodies

# Measurements

- Exercise Tolerance Test
  - Constant speed 2 mph; grade is increased 2% every 2 minutes
  - Peak Walking Time: The time when exercise ceases because of severe claudication
  - Claudication Onset Time: The time when claudication symptoms first occur
- Ankle Brachial Index (ABI)
  - Ratio of SBP measured at ankle to SBP at brachial artery
- Quality of Life Instrument
  - Walking Impairment Questionnaire

# Safety Endpoints

- Death
- Myocardial Infarction
- Stroke
- Critical limb ischemia
- Revascularization procedures
- Cancer

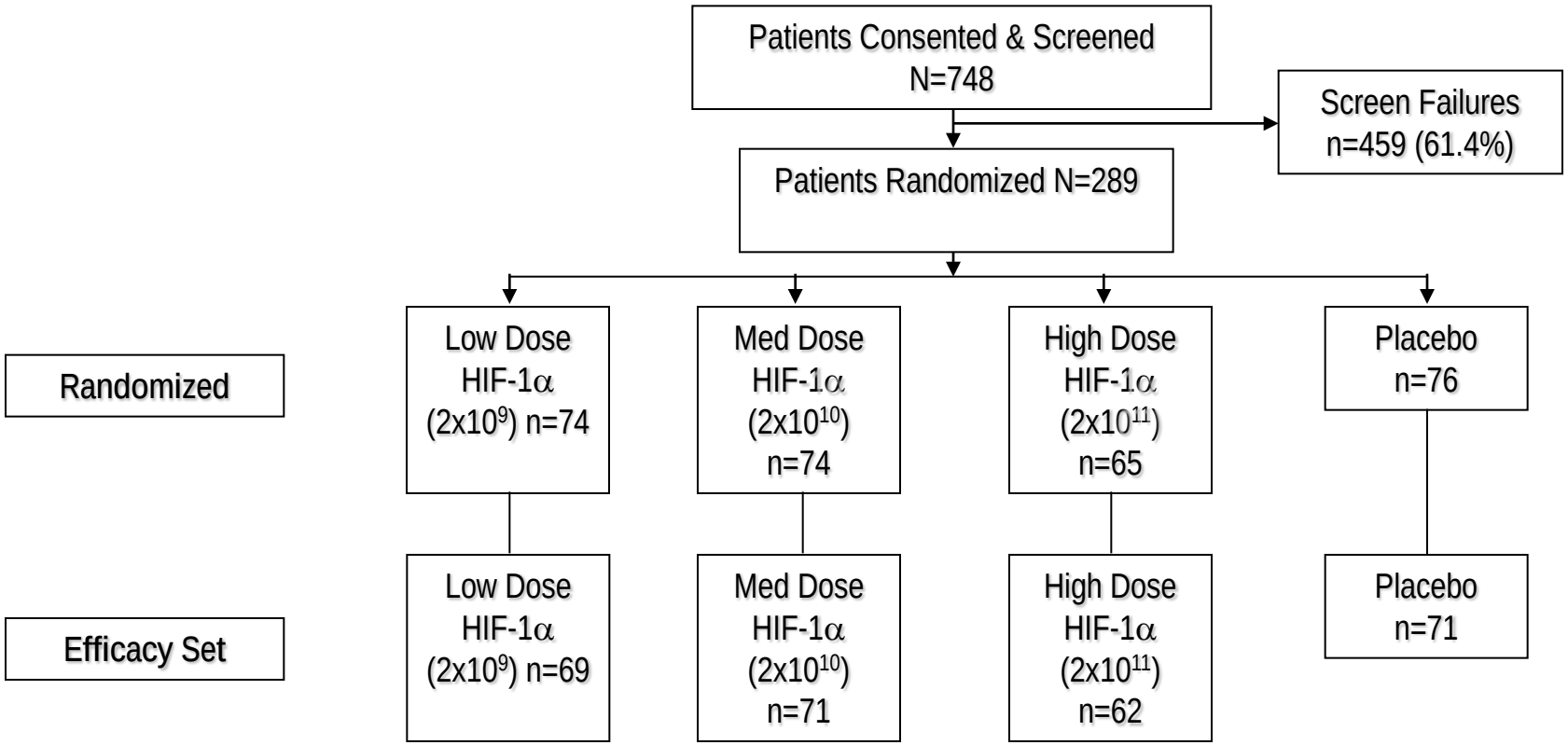
# Cancer Assessment after Randomization

- Chest X-ray- week 52,104
- Stool for occult blood- screening, week 26, 52, 104
- PSA- week 26, 52, 104
- Mammogram- week 52, 104
- Pap and Pelvic Exam- week 52, 104

# Statistical Considerations

- Sample Size Calculation
  - 75 patients randomized to each treatment group provided at least 80% power to detect a treatment effect of 1.5 minutes in PWT at a significance level of 5%.
- Analysis Methods
  - ANCOVA for percent change from baseline
  - Wilcoxon-Mann-Whitney test (for nonparametric data)

# Study Flow Diagram



# Demographics

| Baseline Parameter | | Placebo (N=76) | HIF-1α Low (N=74) | HIF-1α Med (N=74) | HIF-1α High (N=65) |
|---|---|---|---|---|---|
| Age (yrs) | Mean | 66.2 | 65.7 | 68.8 | 66.7 |
| Gender | % Male | 72.4 | 78.4 | 78.4 | 72.3 |
| Ethnic Origin | Caucasian | 63 (82.9) | 64 (86.5) | 68 (91.9) | 56 (86.2) |
| | Black | 9 (11.8) | 8 (10.8) | 4 (5.4) | 8 (12.3) |
| | Asian | 1 (1.3) | 0 | 1 (1.4) | 1 (1.5) |
| | Unknown | 3 (3.9) | 2 (2.7) | 1 (1.4) | 0 |

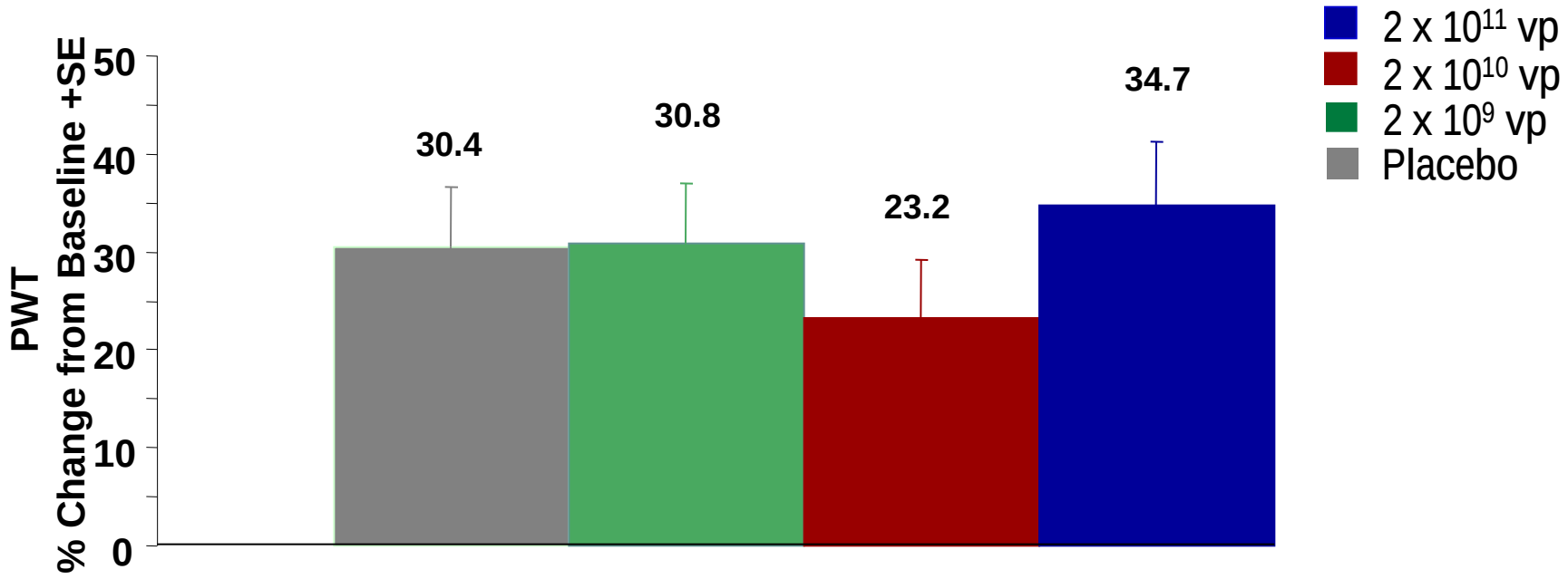
# Baseline Characteristics

| Risk Factor | Placebo (N=76) n (%) | HIF-1α Low (N=74) n (%) | HIF-1α Med (N=74) n (%) | HIF-1α High (N=65) n (%) |
|---|---|---|---|---|
| Type 2 diabetes | 26 (34.2) | 26 (35.1) | 25 (33.8) | 23 (35.4) |
| Hypertension | 67 (88.2) | 68 (91.9) | 68 (91.9) | 59 (90.8) |
| Smoking | 71 (93.4) | 69 (93.2) | 70 (94.6) | 55 (84.6) |
| Hyperlipidemia | 69 (90.8) | 69 (93.2) | 66 (89.2) | 62 (95.4) |

# Baseline Characteristics

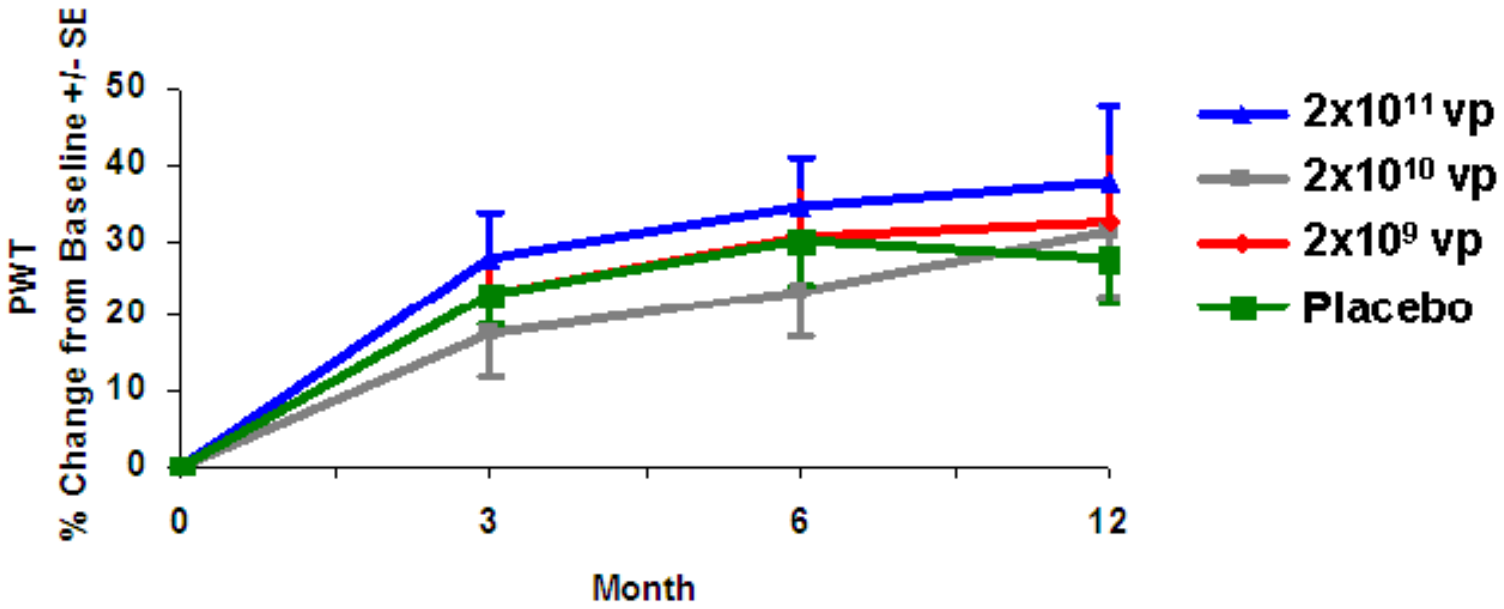
| PAD Measurement | Placebo (N=76) Mean (SD) | HIF-1α Low (N=74) Mean (SD) | HIF-1α Med (N=74) Mean (SD) | HIF-1α High (N=65) Mean (SD) |
|---|---|---|---|---|
| PWT (min) | 4.4 (2.3) | 5.3 (2.7) | 4.7 (2.7) | 5.1 (2.3) |
| COT (min) | 1.8 (1.2) | 1.8 (1.0) | 1.9 (1.4) | 1.9 (1.3) |
| ABI | 0.60 (0.14) | 0.61 (0.14) | 0.57 (0.15) | 0.61 (0.23) |
| WIQ Distance (% max score) | 21 (21) | 22 (25) | 18 (19) | 18 (19) |

# Primary Efficacy Endpoint: Peak Walking Time at 6 months



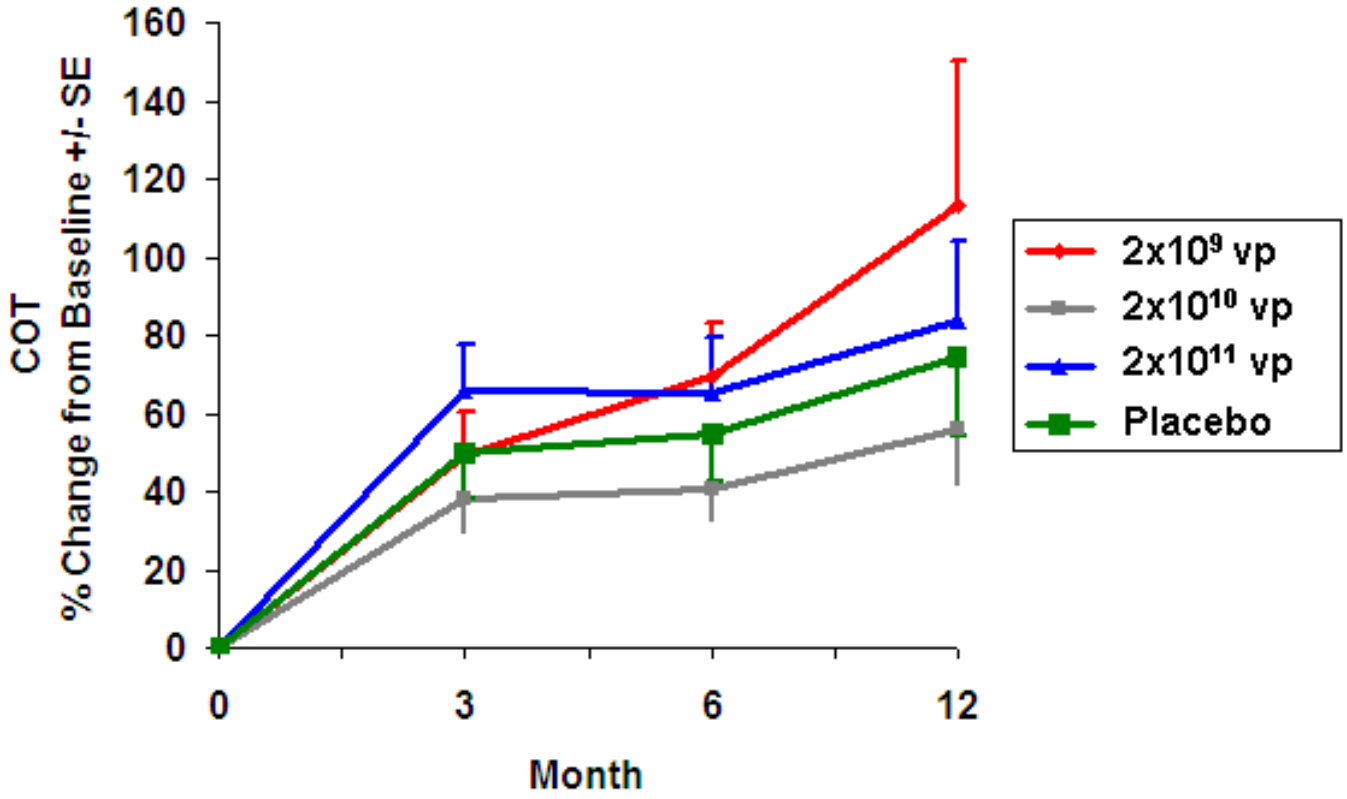

**All p-values NS**

# Secondary Endpoint: Peak Walking Time at 3 & 12 months



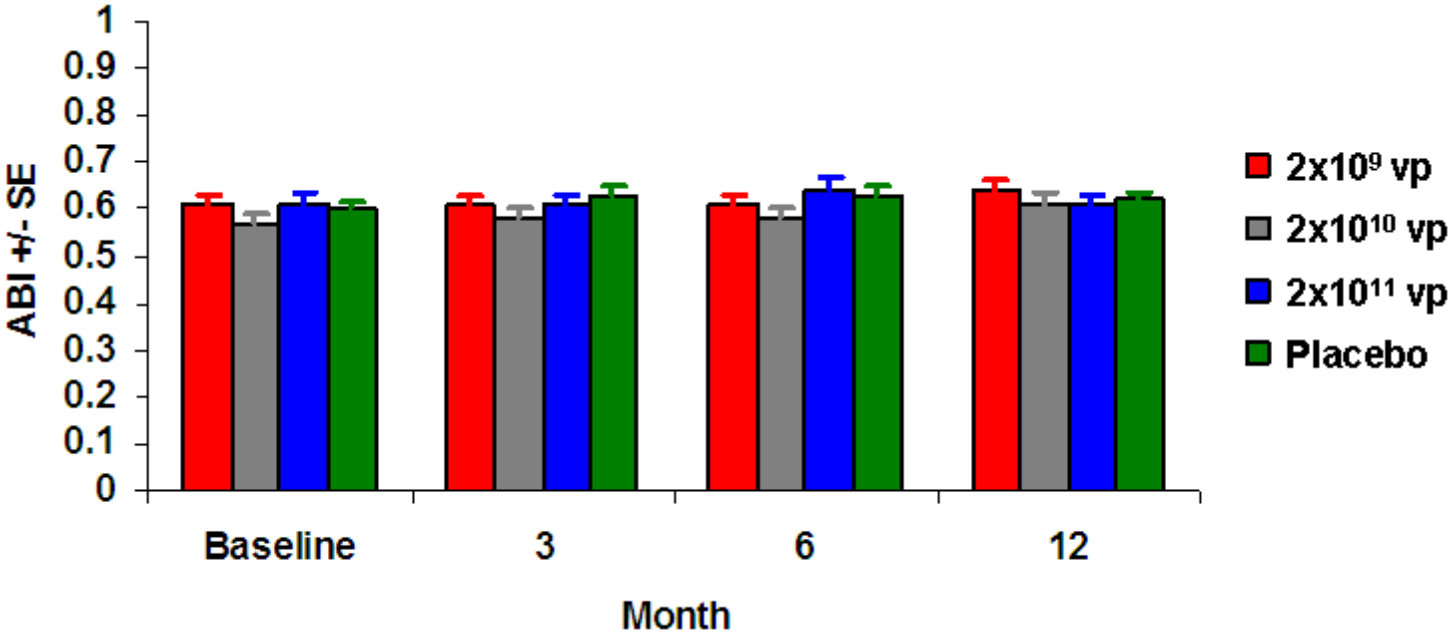

All p-values NS

# Secondary Endpoint: Claudication Onset Time

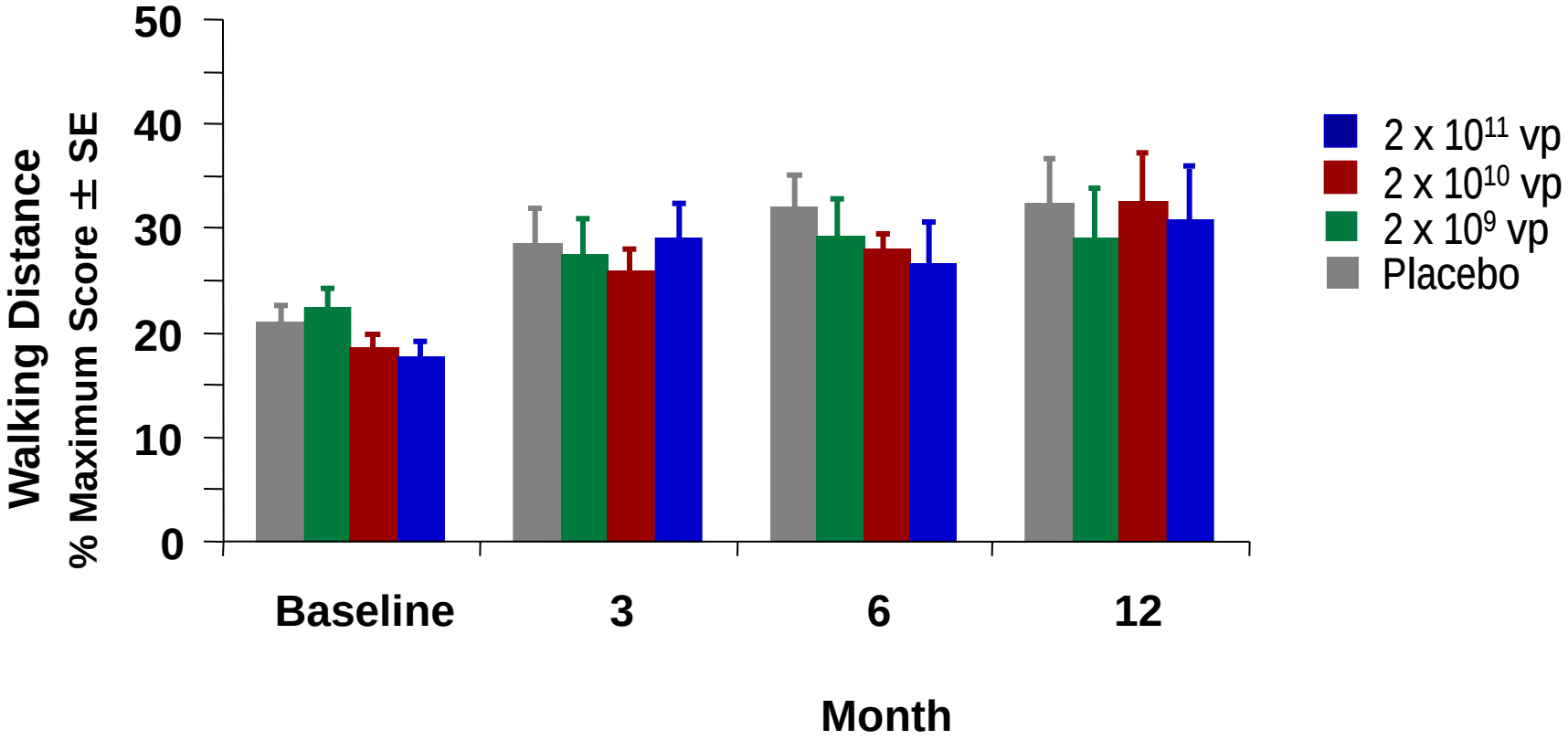



**All p-values NS**

# Ankle Brachial Index: Index Leg



# Walking Impairment Questionnaire: Walking Distance




All p-values NS

# Biomarkers

- Endothelial Progenitor Cells (EPCs)
  - Mean baseline percentage of EPCs in the total cell population was 0.02 — 0.01%
  - No difference from placebo at any time point in groups treated with Ad2/HIF-1α/VP16
- Plasma VEGF
  - The baseline VEGF level for two thirds of the patients in the study (181 out of 275 measured) was below the assay quantitation limit, 31.2 pg/mL.
  - The mean baseline VEGF level among the 94 patients (measurable amounts) was 78.5 — 75.4 pg/ml.
  - No difference from placebo at any time point in groups treated with Ad2/HIF-1α/VP16 treatment groups.

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# Adenovirus Antibodies

- Antibody titer to Adenovirus 2
  - Population mean was 11.7 X $10^3$ with similar values across the randomized groups.
  - Increase in titer in a dose response manner relative to placebo greatest at week 4 and diminished at subsequent time points (all p-values <0.05)
  - No correlations between changes in Ad2 antibody titers and changes in peak walking time at week 26.
- Neutralizing antibody titer
  - Population mean was 2.3 X $10^2$
  - Increase in neutralizing antibody a dose-response manner greatest at week 4 and gradually decline over subsequent time points.
  - Weak correlation of change in titer with and PWT only in lowest dose group weeks 4, 12,and 26 (each r=0.27, p = 0.03)

# Safety Endpoints (Baseline to Week 104)

| | Placebo (N=76) | HIF-1α | | |
|---|---|---|---|---|
| | | Low (N=74) | Med (N=74) | High (N=65) |
| Death | 6 | 5 | 5 | 4 |
| Myocardial Infarction | 4 | 4 | 5 | 5 |
| Stroke | 1 | 3 | 3 | 1 |
| Progression of PAD | 19 | 14 | 13 | 14 |
| Vascular Procedure | 14 | 9 | 12 | 14 |
| Cancer | 4 | 8 | 5 | 8 |

# Cancer Table by Type and Treatment Group

| Cancer Type | Screening Exclusions (N=749) | Treatment Group | | | |
|---|---|---|---|---|---|
| | | Placebo (N=76) n (%) | Ad2/HIF-1α/VP16 | | |
| | | | $2 \times 10^9$ vp (N=73) n (%) | $2 \times 10^{10}$ vp (N=74) n (%) | $2 \times 10^{11}$ vp (N=65) n (%) |
| Leukemia | 1 | 1 (1.3%) | 1 (1.4%) | 0 (0.0%) | 1 (1.5%) |
| Breast cancer | 1 | 1 (1.3%) | 0 (0.0%) | 2 (2.7%) | 1 (1.5%) |
| Laryngeal cancer | 0 | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 1 (1.5%) |
| Lung cancer | 3 | 0 (0.0%) | 1 (1.4%) | 1 (1.4%) | 4 (6.2%) |
| Prostate cancer | 3 | 1 (1.3%) | 2 (2.7%) | 2 (2.7%) | 1 (1.5%) |
| Renal cancer | 1 | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Bladder cancer | 1 | 1 (1.3%) | 1 (1.4%) | 0 (0.0%) | 0 (0.0%) |
| Gastric cancer | 0 | 0 (0.0%) | 2 (2.7%) | 0 (0.0%) | 0 (0.0%) |
| Melanoma | 0 | 0 (0.0%) | 1 (1.4%) | 0 (0.0%) | 0 (0.0%) |
| Total | 10 | 4 (5.3%) | 8 (11.0%) | 5 (6.8%) | 8 (12.3%) |

# Efficacy Summary

Intramuscular administration of Ad2/HIF1$\alpha$/VP16 did not improve:

- Peak Walking Time
- Claudication Onset Time
- Ankle Brachial Index
- Perceived Walking Distance

# Conclusion

Gene therapy with intramuscular administration of HIF-1$\alpha$ is not an effective therapy for patients with PAD and severe intermittent claudication.