NV1FGF/XRP0038

National Institutes of Health (NIH)
Recombinant DNA Advisory Committee (RAC)

124th Meeting
March 8, 2011

Soraya Bekkali, MD
Caroline DeSurmont-Ruchaud, Ph.D

sanofi aventis
Because health matters

NV1FGF/XRP0038

Overview of the project

sanofi aventis

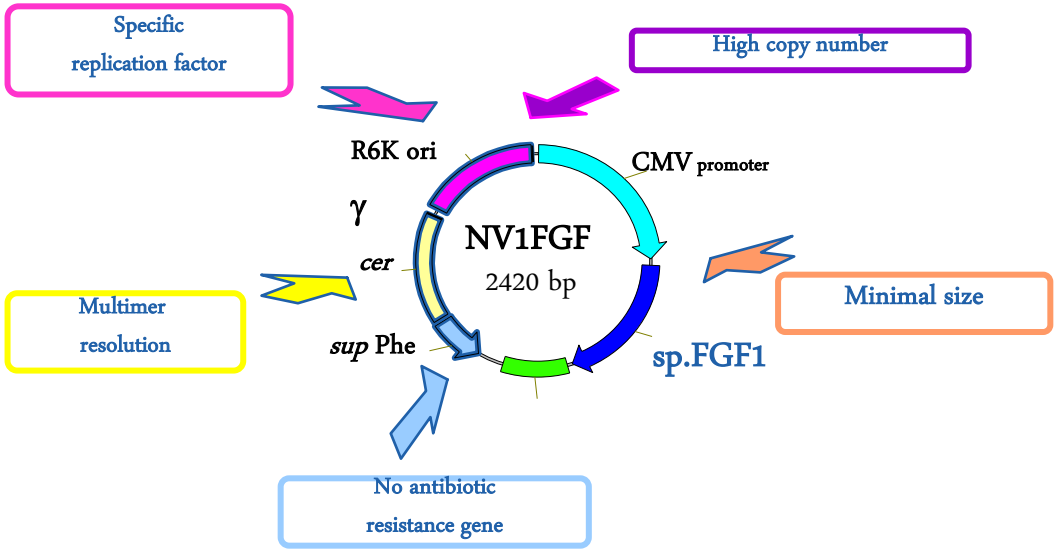
Because health matters

NV1FGF - Product Profile

- Non-viral recombinant DNA plasmid (vector), constructed by inserting a gene encoding for the human acidic fibroblast growth factor (FGF1) into a plasmid backbone with Conditional Origin of Replication (pCOR)

↳replicates only in a specially engineered E. coli strain

- Non-integrative vector

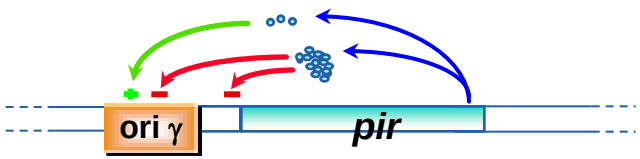


▭: pCOR™ backbone

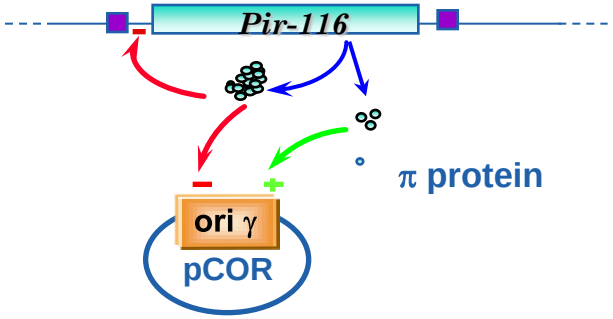
sanofi aventis
Because health matters

pCOR and its host: Safe construct by design to minimize the replication

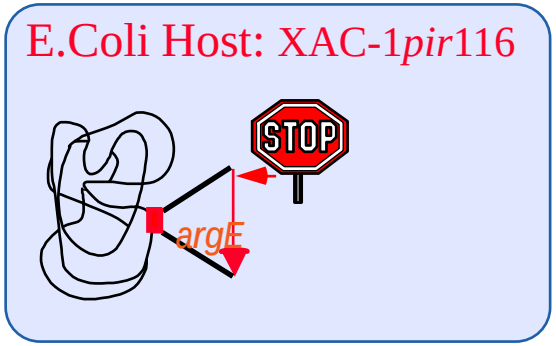
In parental R6K



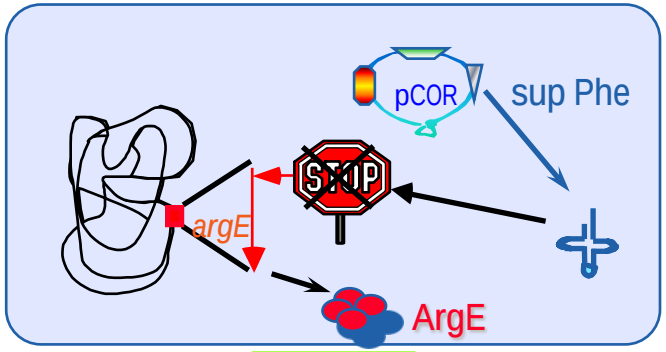
In the genome of E. coli XAC-1



The genetically XAC-1*pir116* pCOR can only replicate in a symbiotic process



Not available to grow in minimal medium lacking arginine



Available to grow in minimal medium lacking arginine

sanofi aventis
Because health matters

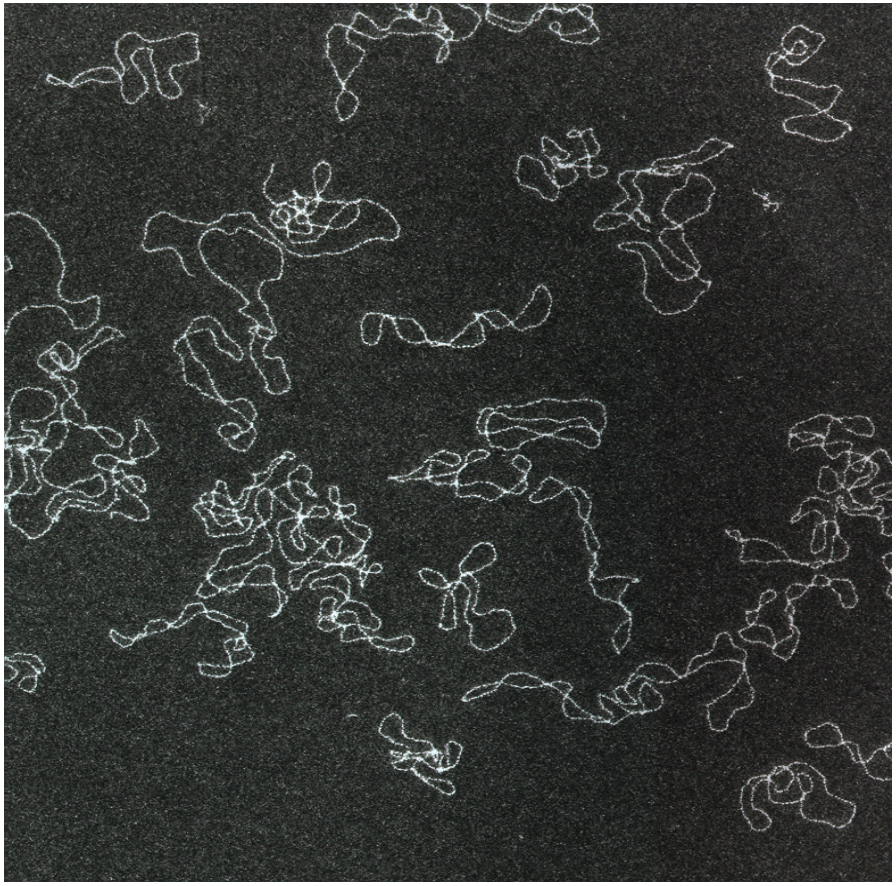
NV1FGF – Drug substance & Drug Product

Drug substance

- Plasmid DNA
- Double strand circular DNA
- Supercoiled form
- 2.42 kbp
- 1.5 million Da

Drug product

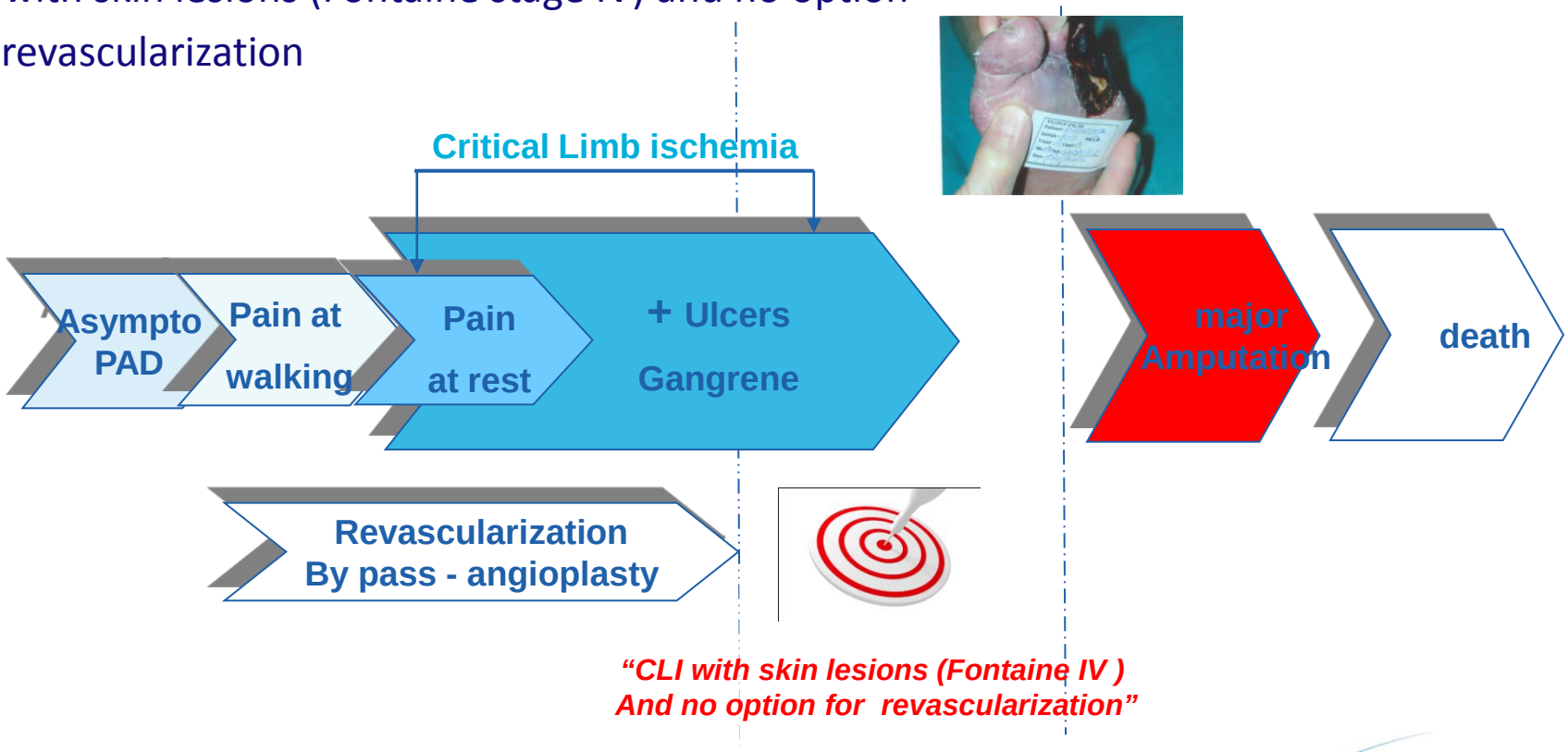
- Solution for injection
- Excipients: water for injection, sodium chloride & trometamol (buffering agent)



sanofi aventis
Because health matters

Target Indication

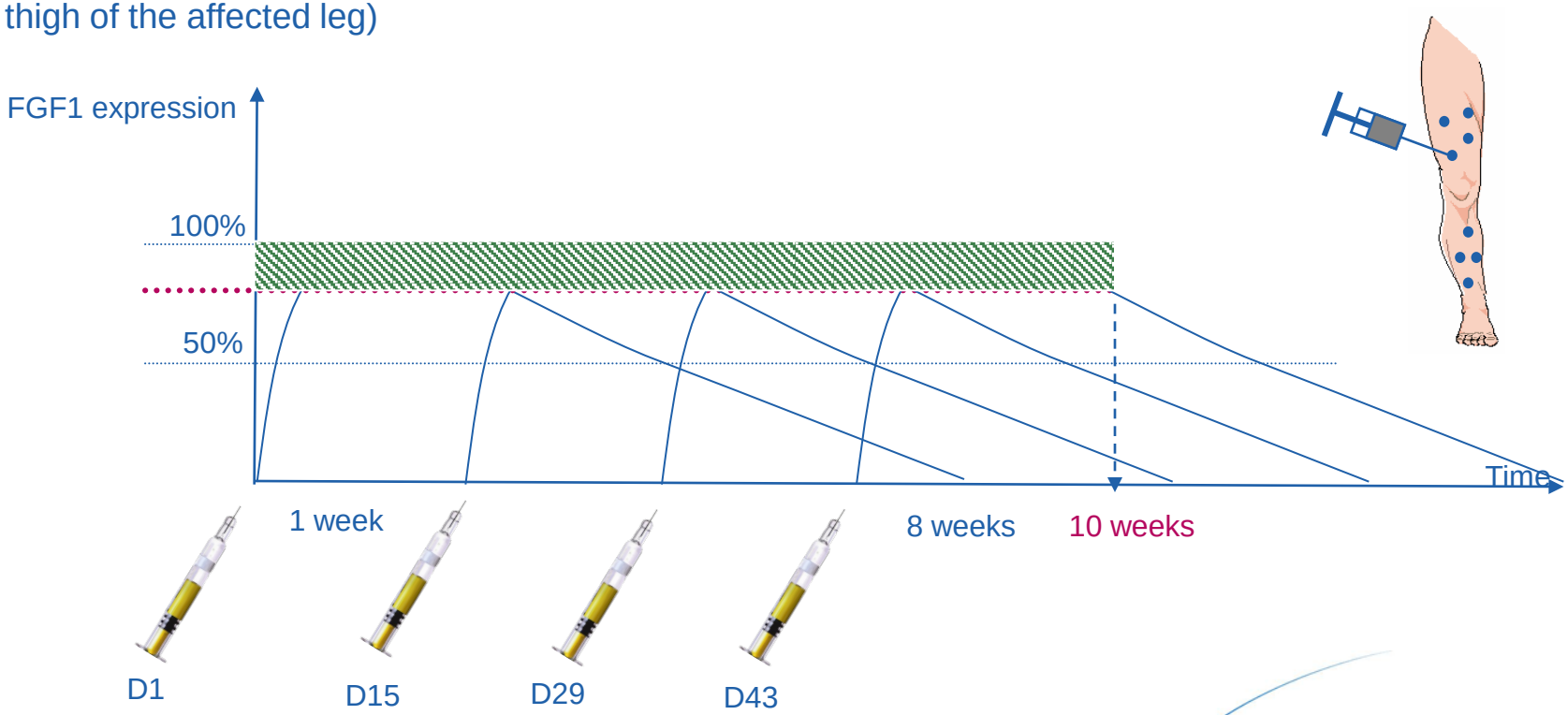
- Therapeutic area: Peripheral Artery Disease (PAD) = (systemic) atherosclerotic stenosis of leg arteries leading to a broad range of symptoms, including critical limb ischemia (CLI)
- Indication: Reduction of major amputation or death in patients with PAD at the stage of CLI with skin lesions (Fontaine stage IV) and no option for revascularization



sanofi aventis
Because health matters

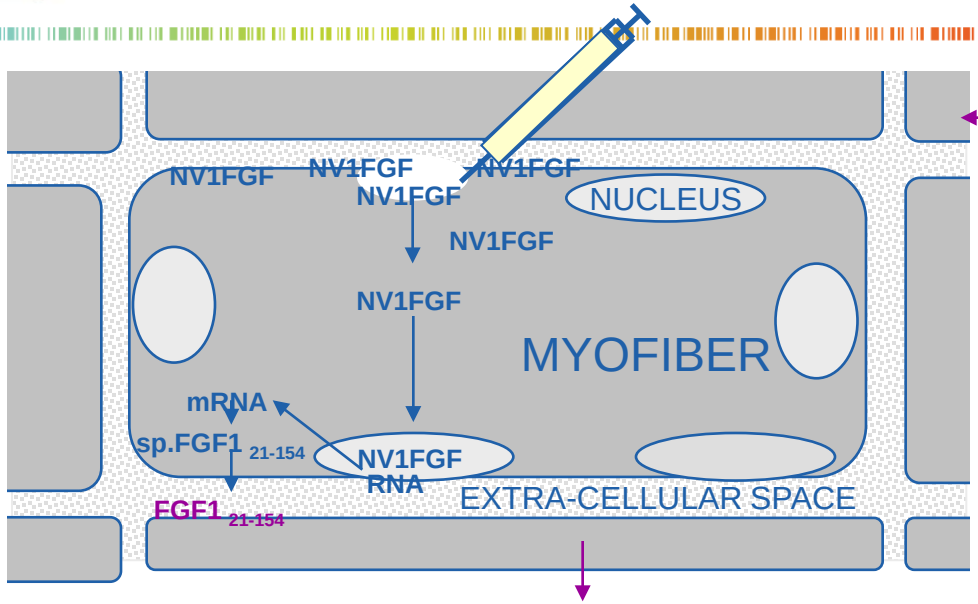
Dose & regimen

- Animal data indicated that after 1 single injection, peak of protein expression was observed at 1 week and no more detected at 8 weeks.
- Expression profile of the FGF1 protein in human was extrapolated from animal PK following single IM injection.
- Each patient received a unique course of treatment in the affected leg . 1 course = 4 administrations of 4 mg, 2 weeks apart (total = 16mg),
- Each of the 4 administrations implies 8 intramuscular injections of 0.5 mg each (4 in the calf, 4 in the thigh of the affected leg)

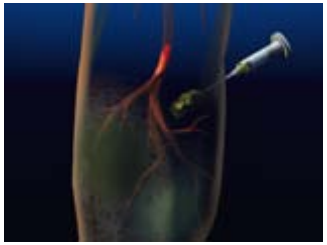


sanofi aventis
Because health matters

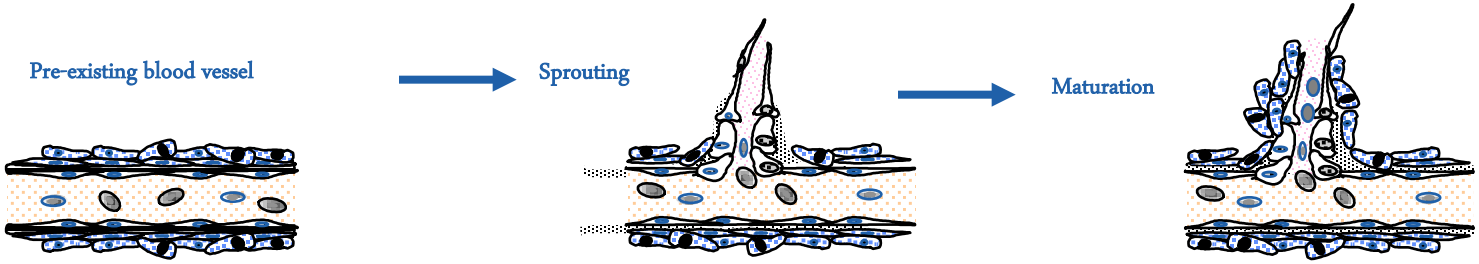
Mechanism of Action



Intramuscular injection of NV1FGF: gene transfer



Expressed FGF1: pro-angiogenic effects



Formation of capillaries

Proliferation, migration, and differentiation of endothelial cells

Maturation to arterioles

Migration and proliferation of SMC, mural cells and pericytes

sanofi aventis
Because health matters

NV1FGF/XRP0038

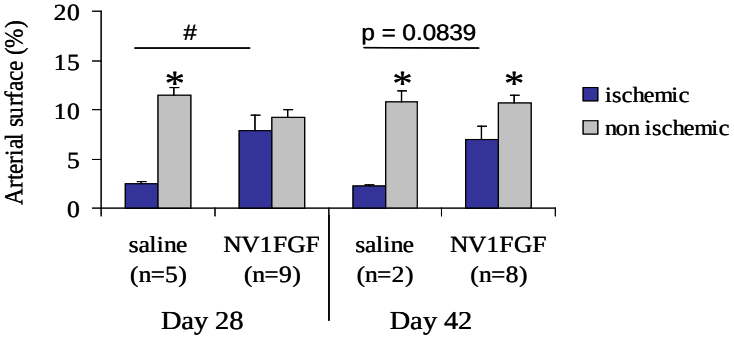
Non-clinical development

sanofi aventis

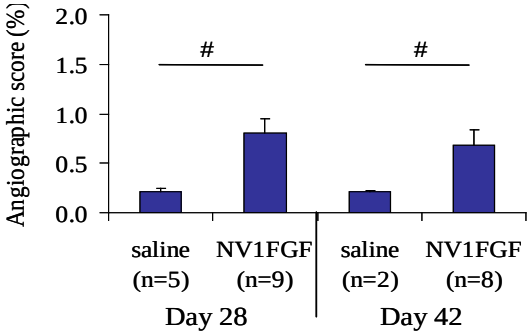
Because health matters

Pharmacology of NV1FGF
Hypercholesterolemic hamster model of PAD: results (1/2)

Arterial surfaces



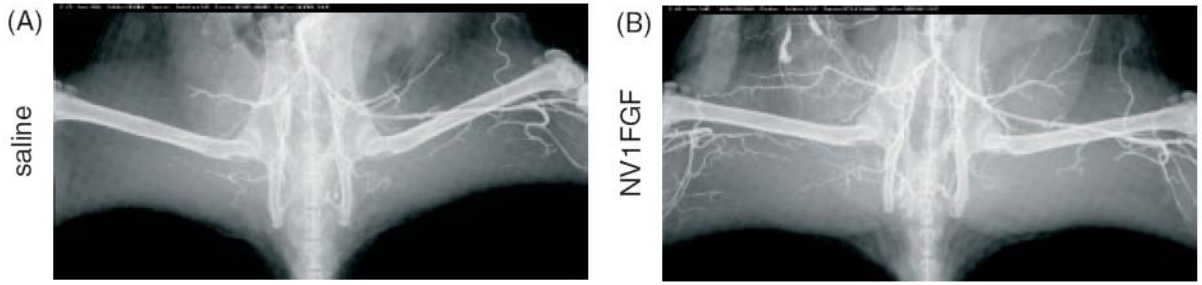
Angiographic Score
(ischemic to non ischemic limb ratio)



# p<0.05 versus saline-treated group

∗ p<0.05 ischemic versus non ischemic limb

Arterial surfaces in hamster treated either with saline or NV1FGF



A. Caron J Gene Med (2004) ***6****, 1033–1045*

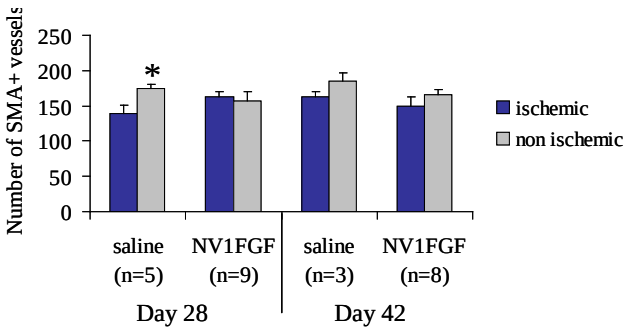
NV1FGF favored the formation of large conductance vessels in hypercholesterolemic hamster with hindlimb ischemia as demonstrated by an increase in angiographic score…

sanofi aventis
Because health matters

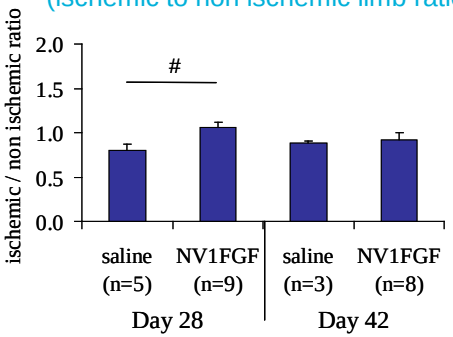
Pharmacology of NV1FGF

NV1FGF in hypercholesterolemic hamster model of PAD: results (2/2)

Number of mature capillaries
(diameter < 50µm)



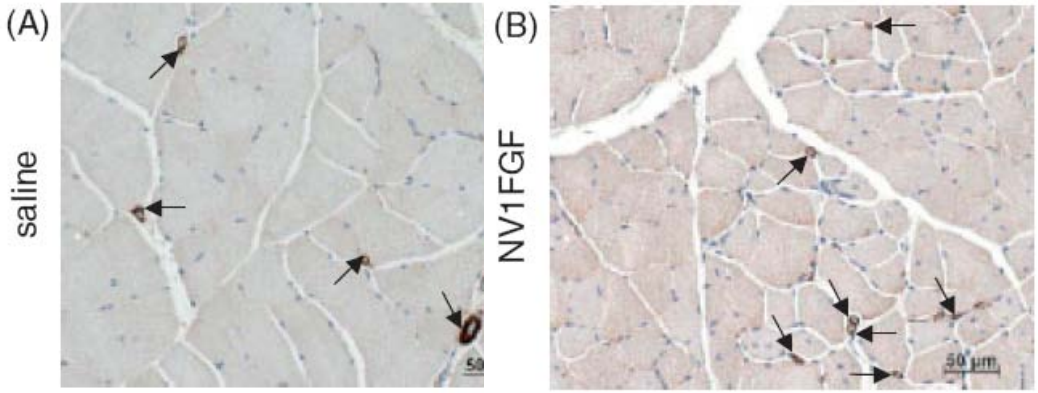
Number of mature capillaries
(ischemic to non ischemic limb ratio)



# p<0.05 versus saline-treated group

∗ p<0.05 ischemic versus non ischemic limb

Number of capillaries in representative hamsters treated either with saline (A) or NV1FGF (B)



*A. Caron J Gene Med (2004) **6**, 1033–1045*

… and NV1FGF induced the development of small conductance vessels suggested by the improvement of the number of arterioles with a diameter < 50 µm

sanofi aventis
Because health matters

Preclinical developement: no evidence of risk

Risks related to:	Theoretical risks	NV1FGF nonclinical studies indicate:
Vectors	▪ For integrating vector >> Insertional mutagenicity	No evidence of risk
Vector & transgene	▪ When broad tissue distribution >> potential toxicity in non target organs, including gonads	No evidence of risk, including in gonads ▪ Local expression, no circulating FGF1 ▪ Non adverse effects, only at injection site ▪ No major risk of germ line transmission
Pro-angiogenic factors	▪ When broad tissue distribution >>Vessels promotion in non target organs (e.g. in eyes, kidney)	No evidence of risk, including in eyes and kidney ▪ Local expression, no circulating FGF1 ▪ No related side effects in distal organs
Growth factors	▪ Tumorigenicity	No evidence of risk ▪ No effect of NV1FGF IM on the growth of pre-existing tumors

sanofi aventis
Because health matters

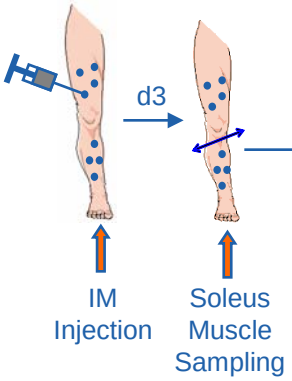
NV1FGF/XRP0038

Clinical Program

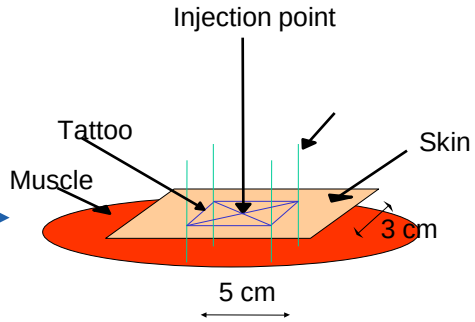
sanofi aventis

Because health matters

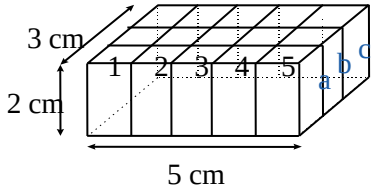
Proof of concept of local transgene expression in CLI amputated patients 1/2



IM: 8 x 0.25 mg
(2.5 ml/injection)

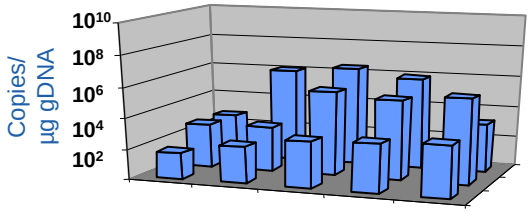


Identification of injection sites



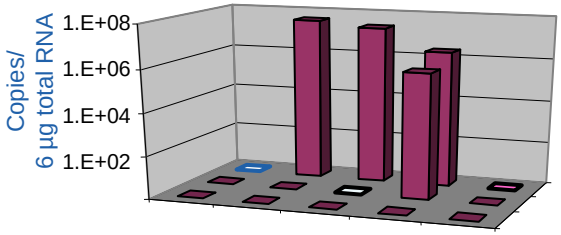
Muscle sampling

DNA



PCR (825 bp;
LOQ: 100 copies/µg gDNA)

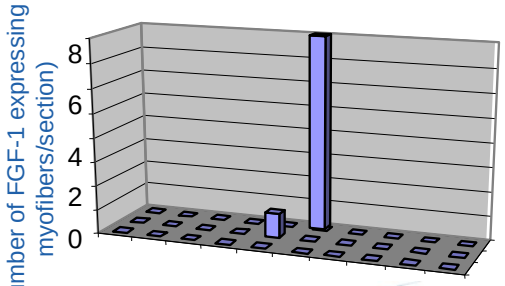
mRNA



RT-PCR (69 bp;
LOQ: 10 copies/µg gDNA)

Study PM105 (n=6 pts)

FGF1



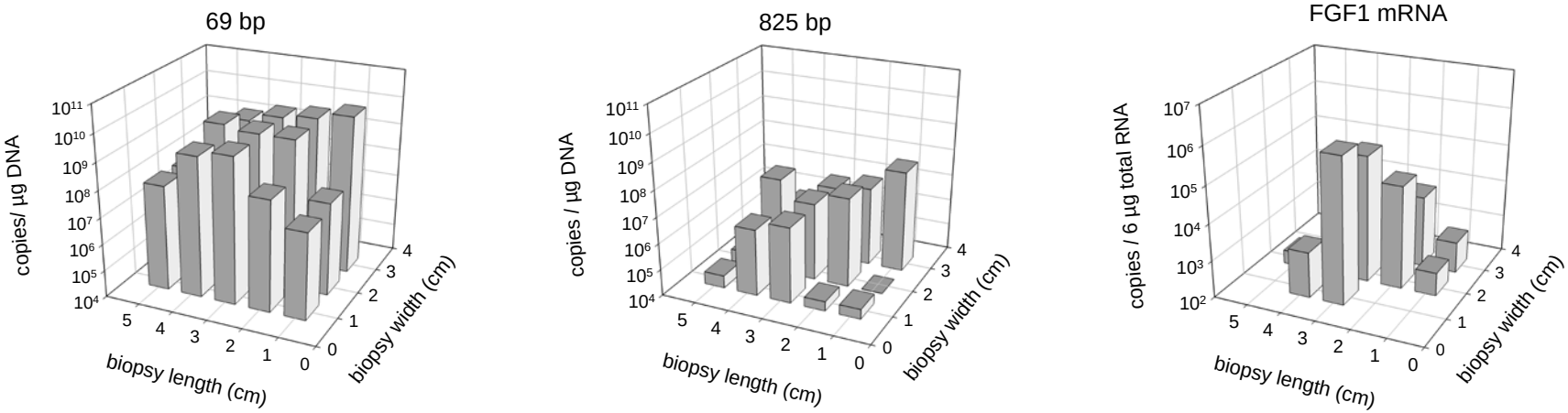
Immunohistochemistry

Baumgartner et al., Molecular therapy 2009

sanofi aventis
Because health matters

Proof of concept of local transgene expression in CLI amputated patients 2/2

Study PM105 - Patient 003/002



- FGF1 transgene expression in muscle was localized to the injection site
- FGF1 not observed in sites distal from injected site
- The PM105 data (local expression and presence of FGF receptors) rationalize the potential for efficacy in humans

Baumgartner et al., Molecular therapy 2009

sanofi aventis
Because health matters

Clinical development program in CLI

Study	Design and Dose Regimen	Patients
PM101/TED10106 (N=51) (EU,US) PK & PD **Efficacy & Safety** TcPO2, ABI, TBI, ulcer size, rest pain, angiography	**Phase 1 study = open-label, non-controlled** single & repeated Dose Escalating IM admin. **Single dose** : 0.5, 1, 2, 4, 8 & 16mg **Two repeated doses** of 0.5, 1, 2, 4 & 8mg at 2-week interval	CLI+ w/wo skin lesions (Fontaine III &IV)
PM105/TED10105 (N=6) (CH,US) PK Plasmid Expression & Biodistribution	**Phase 1 study = double-blind, non-controlled,** multi-center, single IM admin. **Single dose**: 0.5, 2 & 4mg	CLI + major amputation
PM201/DFI6143 (N=125) (EU) Clinical Efficacy and Safety ulcer healing, aggregate ulcer size, amputation, death	**Phase 2 study =** randomized, double-blind, **placebo-controlled,** IM admin. 1 placebo vs 1 NV1FGF arm : 4 x4mg at 2-week interval	CLI+ skin lesions (Fontaine IV)
PM202/DRI6144 (N=71) (US) PK & PD (TCPO2) and safety	**Phase 2 study =** randomized, double-blind, **placebo-controlled,** IM admin. of different doses & regimens 1 placebo vs 5 NV1FGF arms: 2x8, 4x0.5, 4x2, 4x4 mg at 2 weeks intervals, and 2x8 mg at 4-week interval	CLI +skin lesions (Fontaine IV)
TAMARIS study (EFC6145): (N525) (WW) Pivotal efficacy and safety study	**Phase 3study =** randomized, double-blind, **placebo-controlled, 4** IM admin. of 4x4 mg at 2 weeks intervals.	CLI +skin lesions (Fontaine IV)

sanofi aventis
Because health matters

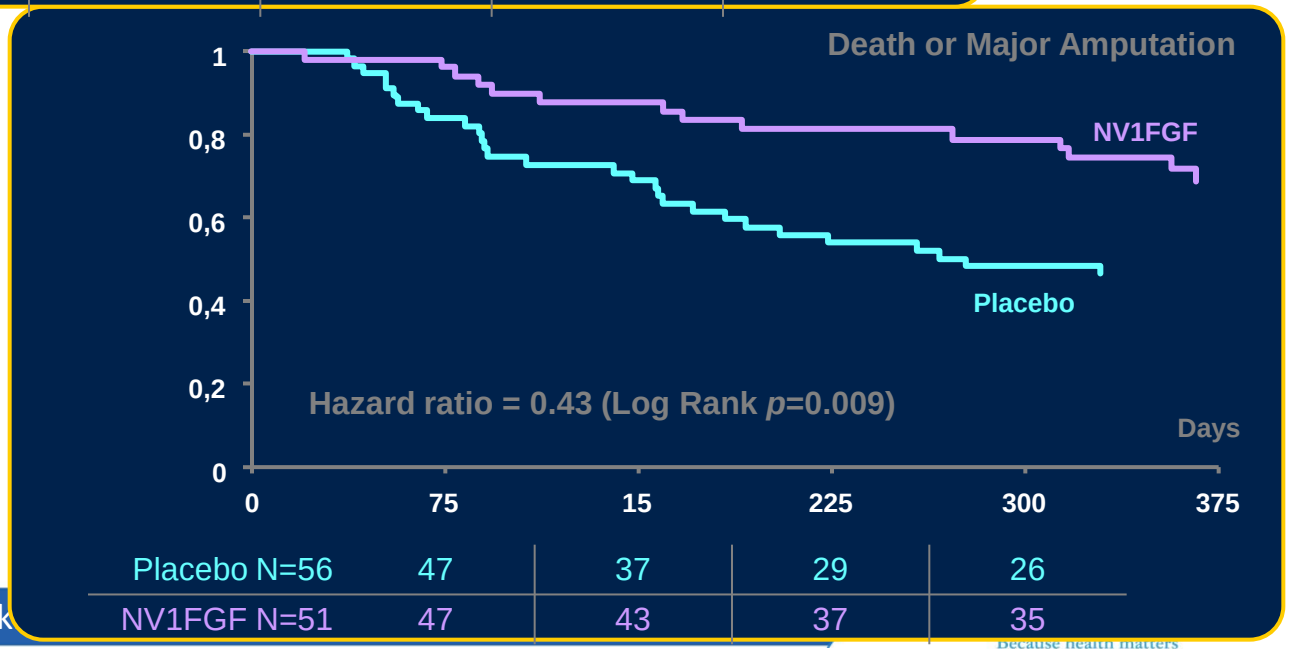
NV1FGF Phase 2 (TALISMAN study)

- **125 patients with CLI and ischemic ulcers (Fontaine IV) unsuitable for revascularization**
- **Randomized to 16mg of NV1FGF or placebo**
  (4 mg administered as 8 IM injections every two weeks for a total of 6 weeks)
- **Primary endpoint = Complete ulcer healing at 25 weeks**
- **Secondary endpoints = major amputations, death, amputation free survival at 12 months**

sanofi aventis
Because health matters

NV1FGF Phase 2 results

	Placebo (N=56)	NV1FGF (N=51)	Hazard ratio	*P* value
Primary end point				
Complete healing of at least one ulcer selected at baseline at week 25	8 (14.3)	10 (19.6)	-	0.514*
Secondary end points over 52 weeks				
Amputation rate				
All	31 (55.4)	19 (37.3)	0.498	0.015[b]
Major	19 (33.9)	8 (15.7)	0.371	0.015[b]
Death rate	13 (23 *2*)	6 (11.8)	0.460	0.105[b]
Combined major amputation and death rates	29 (51.8)	14 (27.4)	0.435	0.009[b]



Nikol, S et Al. Mol Ther 2008;16:972-8

NV1FGF Gene Therapy on Amputation-Free Survival in Critical Limb Ischemia

Phase 3 Randomized Double-Blind Placebo-Controlled Trial

TAMARIS results

sanofi aventis

Because health matters

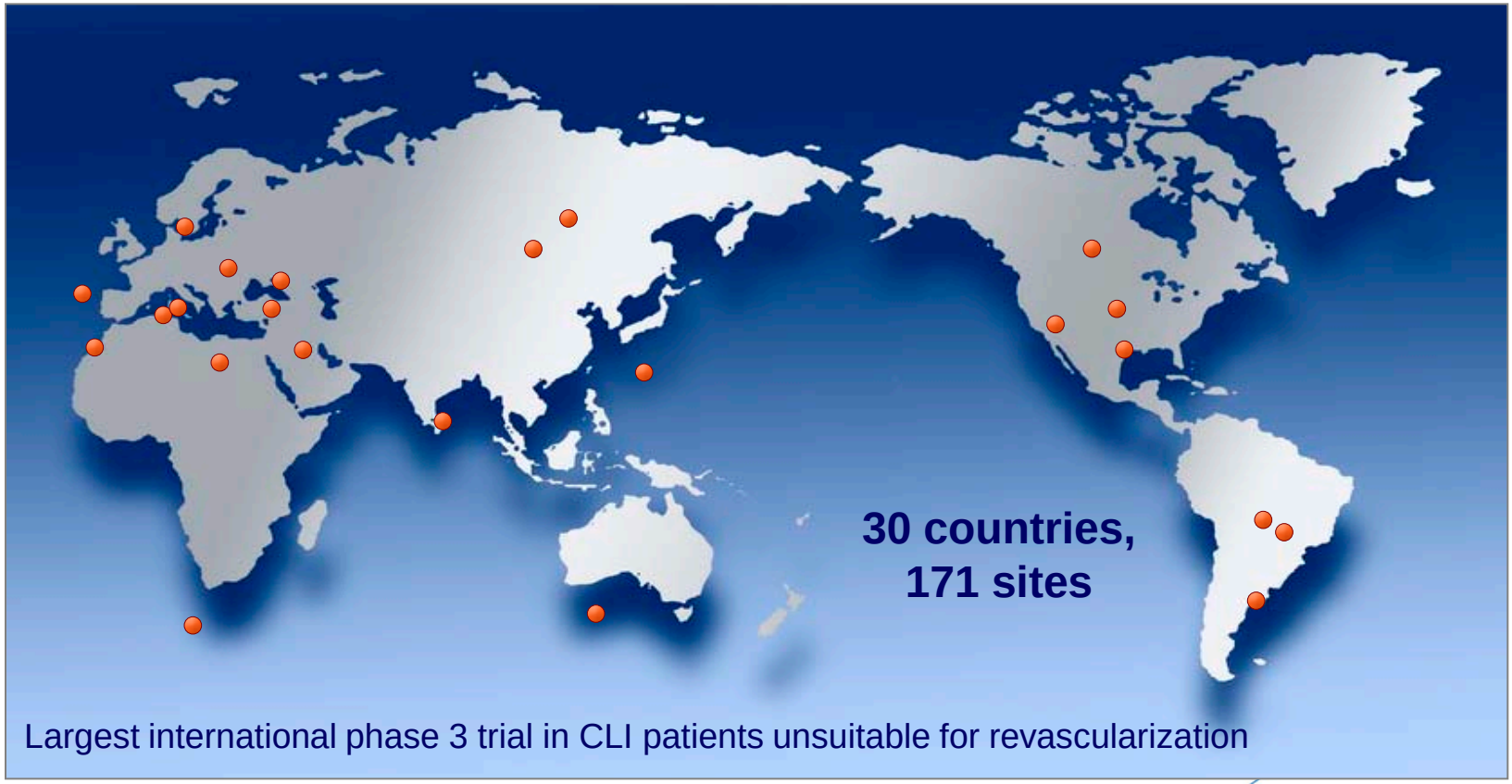
NV1FGF Phase 3: TAMARIS Study

525 CLI patients with skin lesions (Fontaine IV), unsuitable for standard revascularization

30 countries (US, Canada, South America, Europe, Asia, Japan, Australia)

Randomized to NV1FGF 4mg x 4 every two weeks or placebo

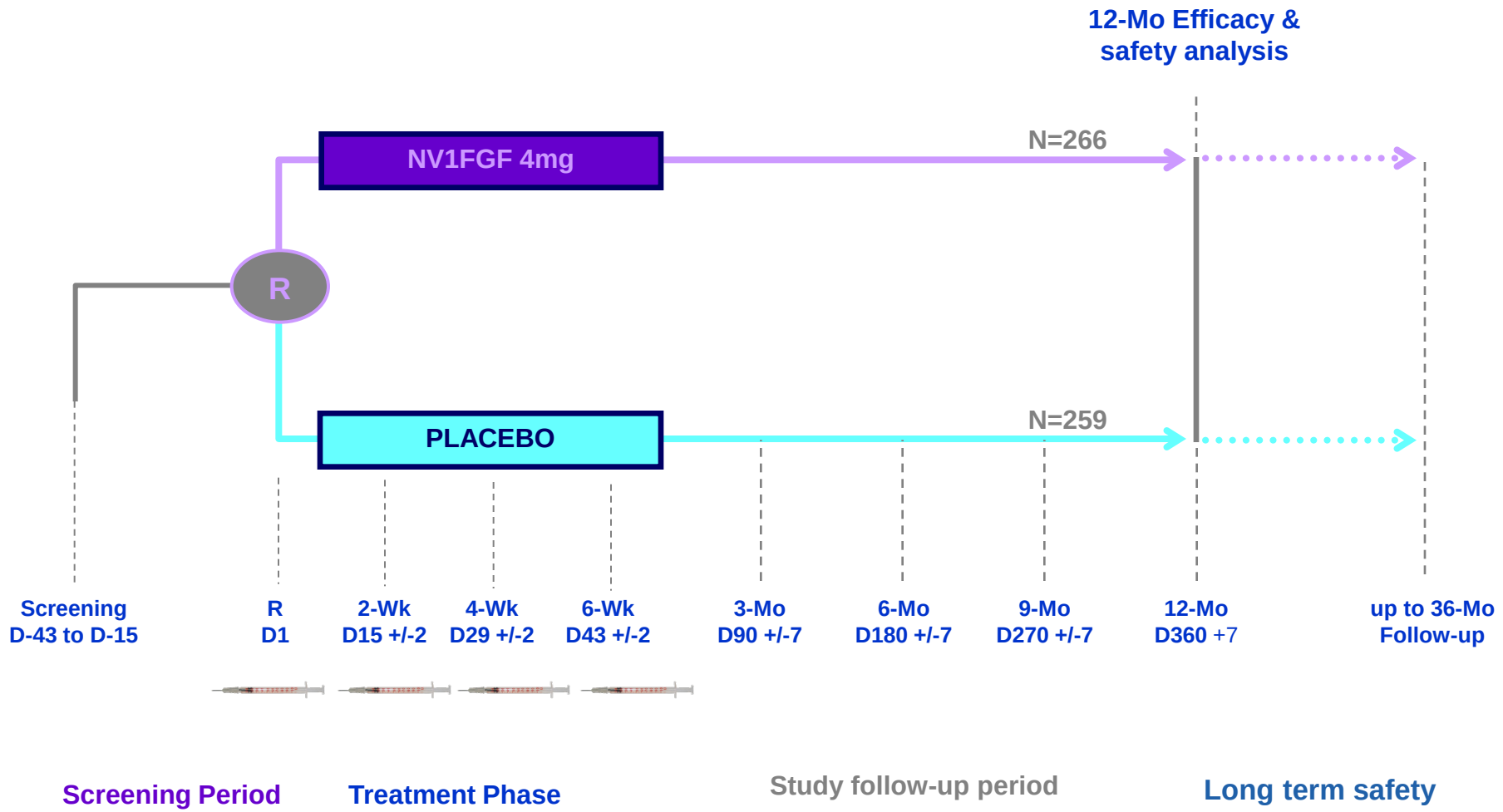
Double blind assessment, stratification by country and diabetes



Largest international phase 3 trial in CLI patients unsuitable for revascularization

sanofi aventis
Because health matters

TAMARIS trial design
(replicates Phase 2 including dosing)



sanofi aventis
Because health matters

Endpoints

Primary endpoint:

- First occurrence of major amputation (above the ankle) of the treated leg or death from any cause over 12 months

Secondary endpoints:

- All amputations
- Death
- Ulcer healing
- Pain relief
- Functional status

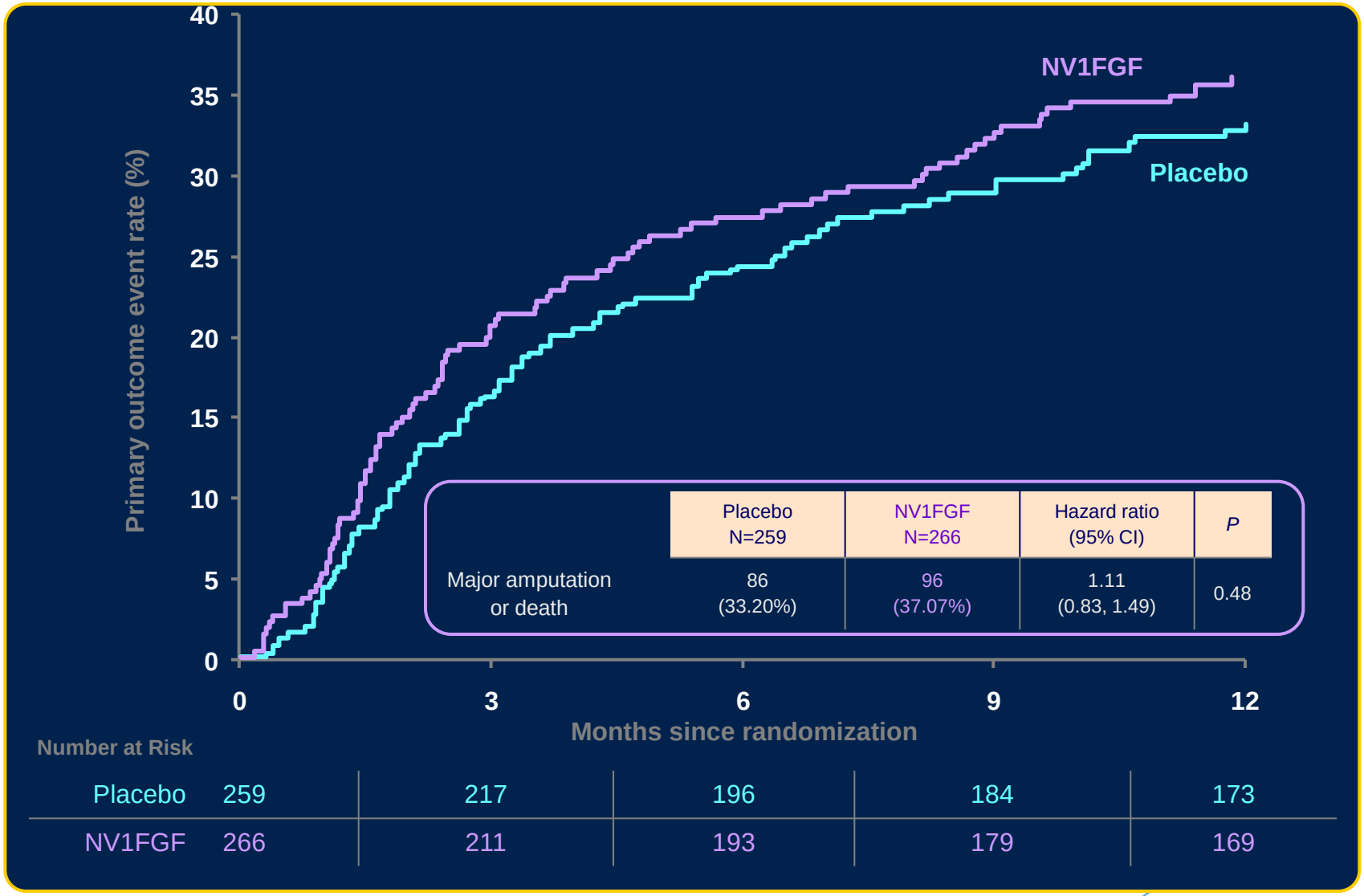
sanofi aventis
Because health matters

Baseline Demographics

	Placebo	NV1FGF
Number	259	266
Mean Age (Range)	69 (50-90)	71 (50-95)
Male	181 (69.9%)	184 (69.2%)
Current/Former smoker	165 (63.7%)	156 (58.7%)
Hypertension	213 (82.2%)	233 (87.6%)
Hyperlipidemia	153 (59.1%)	164 (61.7%)
Diabetes	141 (54.4%)	139 (52.3%)
Angina/unstable angina	91 (35.1%)	72 (27.1%)
Myocardial infarction	69 (26.6%)	61 (22.9%)
Atrial fibrillation	53 (20.5%)	46 (17.3%)
Stroke	38 (14.7%)	40 (15.0%)
Congestive heart failure	39 (15.1%)	53 (19.9%)

sanofi aventis
Because health matters

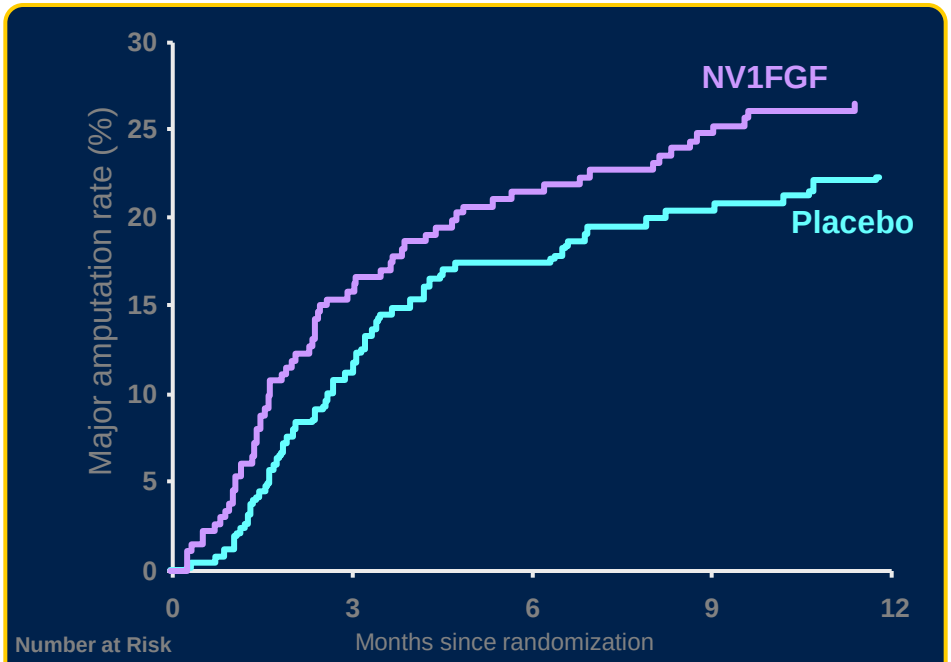
Primary Endpoint
Time to Major Amputation or Death



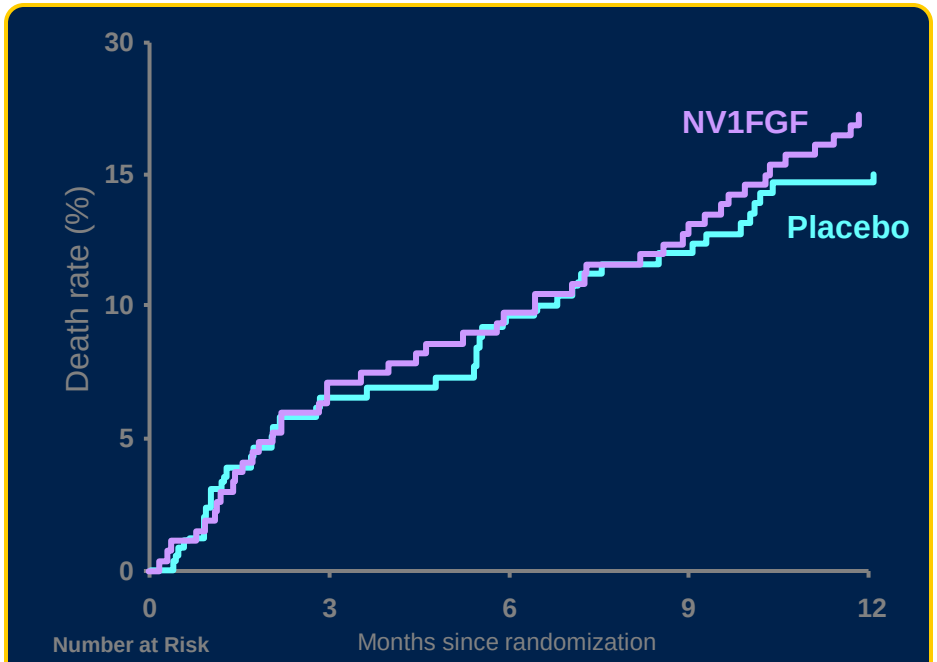
	Placebo N=259	NV1FGF N=266	Hazard ratio (95% CI)	*P*
Major amputation or death	86 (33.20%)	96 (37.07%)	1.11 (0.83, 1.49)	0.48

sanofi aventis
Because health matters

Secondary Endpoints
Time to Major Amputation & time to Death



Number at Risk					
Placebo	259	217	196	184	173
NV1FGF	266	211	193	179	169



Number at Risk					
Placebo	259	242	234	228	220
XRP0038	266	247	240	231	219

	Placebo N=259	NV1FGF N=266	Hazard ratio (95% CI)	*P*
Major amputation	55 (21.24%)	67 (25.87%)	1.20 (0.84, 1.72)	0.31
Death	39 (15.06%)	46 (17.76%)	1.15 (0.75, 1.76)	0.53

sanofi aventis
Because health matters

TAMARIS Subgroup analysis (by diabetes stratum)

	With diabetes		Without diabetes	
	Placebo (N=140)	NV1FGF **(N=137)**	Placebo (N=119)	NV1FGF **(N=129)**
Primary outcome: major amputation or death	**45 (32.14%)**	**52 (37.96%)**	**41 (34.4%)**	**44 (34.1%)**
Death	**21 (15.00%)**	**17 (12.41%)**	**10 (8.4%)**	**12 (9.3%)**
First major amputation on the treated leg	**24 (17.14%)**	**35 (25.55%)**	**31 (26.1%)**	**32 (24.9%)**
Log rank p-value	**0.3431**		**0.9771**	
Hazard ratio (95% CI)	**1.21 (0.81, 1.81)**		**1.01 (0.66, 1.54)**	

sanofi aventis
Because health matters

Extent of exposure

Extent of exposure	Placebo N=257	NV1FGF N=266
1 administration	5	10
2 administrations	12	13
3 administrations	15	17
4 administrations (complete course)	225 (87.5%)	226 (85.0%)

sanofi aventis
Because health matters

Safety at 1 year – plan for long term follow-up

Overall safety	Placebo N=257	NV1FGF N=266
Adverse events	205 (79.8%)	214 (80.5%)
Serious adverse events	151 (58.8%)	163 (61.3%)
Adverse event leading to death	36 (14.0%)	35 (13.2%)
Adverse event leading to permanent treatment discontinuation*	15 (5.8%)	24 (9.0%)

*Most patients discontinued due to infections and infestations 2.7% Placebo vs 4.5% NV1FGF or vascular disorder (mainly peripheral ischemia) 1.9% placebo vs 1.9% (NV1FGF)

- Of note, as per EMA guidance for long term follow-up of patients treated with gene therapy products, study patients will be followed up to 3 years as per protocol
- Safety reporting via INDAR and ASR

sanofi aventis
Because health matters

Safety – Events of special interest for a growth factor/gene therapy

	Placebo N=257	NV1FGF N=266	Relative risk (95% CI)	*P*
Ischemic heart disease*	26 (10.1%)	27 (10.2%)	1.00 (0.60, 1.67)	1.00
Ischemic cerebrovascular conditions*	10 (3.9%)	11 (4.1%)	1.07 (0.46 to 2.46)	1.00
Retinal disorders**	15 (5.8%)	11 (4.1%)	0.71 (0.33, 1.51)	0.42
Renal impairment*	15 (5.8%)	20 (7.5%)	1.29 (0.67, 2.46)	0.49
Malignant neoplasm*	4 (1.6%)	7 (2.6%)	1.69 (0.50, 5.71)	0.55
Injection site disorders	3 (1.2%)	3 (1.1%)	0.97 (0.20 to 4.74)	1.00
Peripheral leg edema	17 (6.6%)	22 (8.3%)	1.25 (0.70 to 2.30)	0.51
Immunological disorders*	6 (2.3%)	4 (1.5%)	0.64 (0.18 to 2.26)	0.54

*MedDRA SMQ

sanofi aventis
Because health matters

Safety - Malignant neoplasms at 1 year

	Placebo N=257	NV1FGF N=266
Malignant neoplasm (MedDRA SMQ)	**4 (1.6%)**	**7 (2.6%)**
Colon cancer	1	
Gastric cancer		1
Hepatic cancer metastatic	1	
Tongue neoplasm malignant		1
Mediastinum neoplasm	1	
Squamous cell carcinoma		1
Non small cell lung cancer		1
small cell lung cancer	1	
Salivary gland cancer	1	
Basal cell carcinoma (skin)	1	1
Squamous cell carcinoma (skin)		2
Thyroid cancer	1	
Urinary carcinoma		1

sanofi aventis
Because health matters

NV1FGF/XRP0038

Debriefing on TAMARIS outcome
Clinical Investigations

sanofi aventis

Because health matters

Development Story line

Preclinical

- Proof of concept of the angiogenic effect in two animal models
- A complete toxicology and bio-distribution package, with no toxicity findings at doses up to 43 times the intended clinical dose.

Clinical

- Proof of concept of transgene expression in CLI patients after IM administration
- No major safety risk associated with the IM administration of various doses (0.5mg to 8mg x 4) in patients. No immunogenic response.
- The phase 2 dose finding study (PM202 study) was non conclusive. Doses tested ranged from 0.5mg once to 4mgx4 [i.e. TAMARIS dose/regimen].
- Statistically significant relative risk reduction (63%) of major amputations in the phase 2 study (TALISMAN) as a secondary endpoint where ≈ 120 patients received the same dose and same regimen (4mg x 4).
- Set-up of randomized placebo controlled phase 3 study, n >500 patients, receiving 4 mg x 4, with major amputation or death as primary endpoint

sanofi aventis
Because health matters

In depth analysis of TAMARIS data

- **In depth analysis of Phase 3 results was performed with the help of TAMARIS steering committee:**
  - No indication of efficacy suggested by in depth analyzes of TAMARIS data :
    - Secondary analysis (hemodynamic parameters, leg pressures, etc)
    - subgroup analyses by demographics, medical history, geographic regions, presence of gangrene, renal impairment stage, etc
    - Sub-studies (TcPo2, ulcer size measurement, and Skin perfusion pressure)
  - TAMARIS study design and rigorous conduct make the data fully reliable and this trial conclusive.
    - Size of the trial, appropriate statistical power
    - Sponsor's oversight (including audits)
    - Steering Committee monthly oversight
    - Adjudication committee
    - No patient lost to follow-up

sanofi aventis
Because health matters

TAMARIS vs TALISMAN studies
Design

- **Patients' selection criteria, dose and schedule of administration, follow-up duration**
  - Identical between Phase 2 (TALISMAN) and Phase 3 (TAMARIS) trial

- **Geographic regions**
  - TALISMAN performed in 6 European Countries
  - TAMARIS performed in 30 worldwide countries
    - Subgroup analysis by region (continent) show no statistical difference
    - Subgroup analysis focusing on the TALISMAN Phase 2 countries/sites show no difference

- **More robust design of the Phase 3 TAMARIS trial**
  - Larger sample size, with time to major amputation or death as primary endpoint
  - Randomization stratified by country and diabetes status
  - Centralized adjudication of - Patients' unsuitability for revascularization (eligibility) & justification for amputation (primary endpoint)

sanofi aventis
Because health matters

TAMARIS vs TALISMAN studies
study populations

- Similar baseline characteristics in both studies except for:
  - Diabetes (10% more patients in TAMARIS)
  - Severe renal insufficiency (15% more in TAMARIS)
  - However subgroup analyses by diabetes or renal insufficiency status do not show any difference

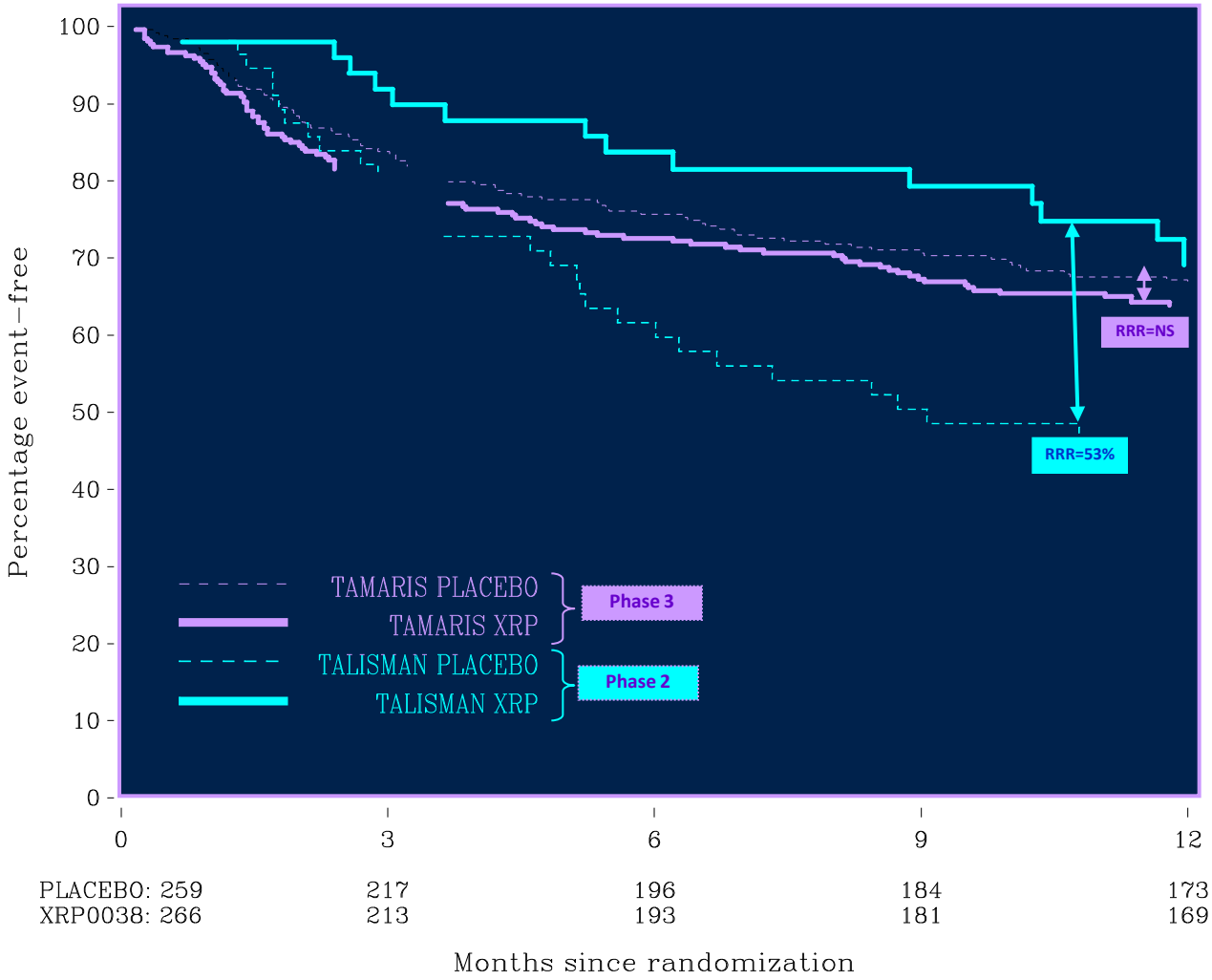
  ↳ **Overall, these differences were considered minor by the steering committee and can not explain the difference in study outcomes between Phase 2 and 3 trials**

sanofi aventis
Because health matters

TAMARIS vs TALISMAN studies
Placebo event rate

Major amputation or death rate at 1 year: Placebo rate over 50% in Phase 2 TALISMAN study versus 33% in Phase 3 TAMARIS

- Compared event rates in both studies for the active arm, 35% and 37% respectively.
- It is unlikely that medical management of vascular risk had improved over the past 4 years between the two studies
- High variability of placebo rate among subgroup analysis



Time to first major amputation or death TAMARIS and TALISMAN event rates (Placebo and XRP0038)

sanofi aventis
Because health matters

TAMARIS vs TALISMAN studies Conclusion

- **When looking at TAMARIS subgroup analyses (e.g. by region, by diabetes), the placebo event rate (of major amputation or death) displays a large variability**
  - (ranging from 20% in Latin America (n=67) , 32% in western Europe (n=179) to 48.5% in North America (n=63)
  - Suggesting that the placebo event rate of 50% in the Phase 2 TALISMAN study was only one possible point estimate observed in relatively small sample patients (n≈ 55 per group in TALISMAN study) among others possible samples in this CLI population.

↳ **The significant difference in the secondary endpoint (major amputation or death rate) observed in the small TALISMAN phase 2 trial between NV1FGF and placebo arm is probably a chance finding.**

sanofi aventis
Because health matters

NV1FGF/XRP0038

Debriefing on TAMARIS outcome
Study Drug Investigations

sanofi aventis

Because health matters

Root causes related to investigational product

- **misallocation of study treatment during Phase 3 trial**
  - verification of packaging batch numbers
  - verification of the randomization list
  - re-test by UV of IP (XRP0038 and placebo vials) retrieved from clinical sites
    - The content of all the tamaris treatment kits tested (133 vials in total) corresponds to what was expected (XRP0038 or placebo)

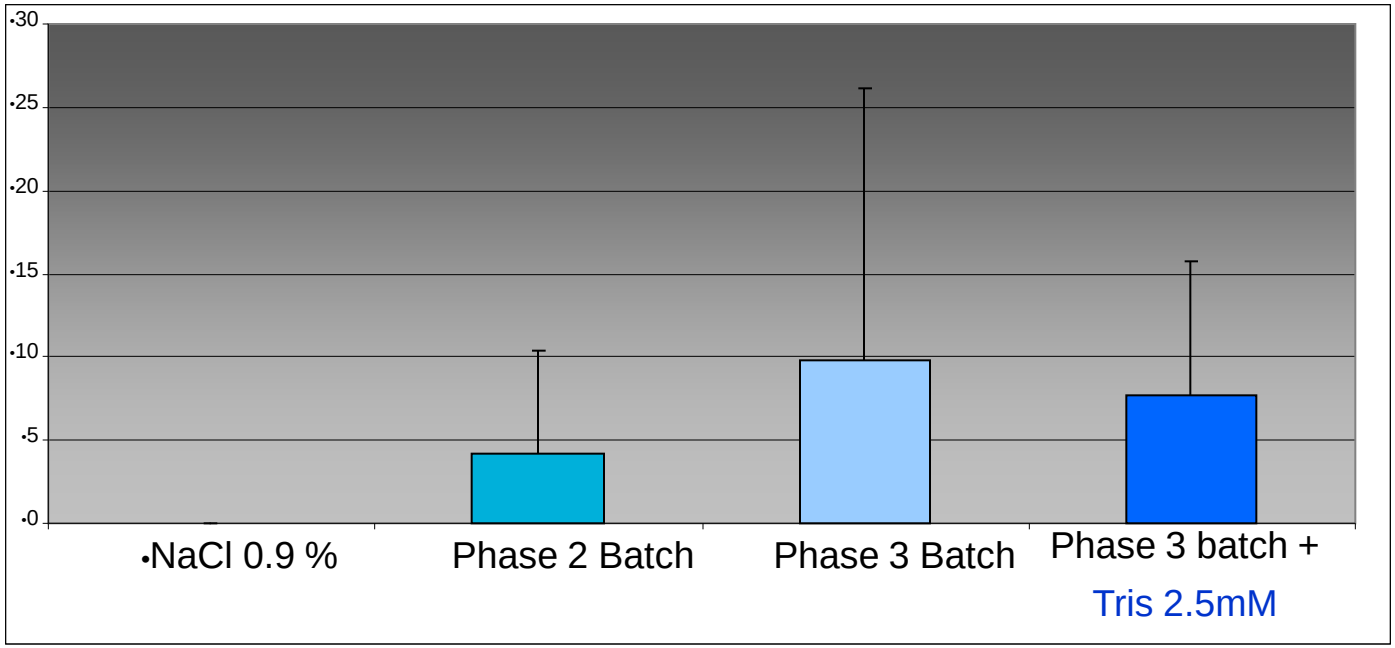
↳ **treatment allocation process occurred with no errors**

- **manufacturing changes between Phase 2 and 3 lots**
  - Changes were introduced between Phase 2 and Phase 3 in drug substance manufacturing and drug product formulation
  - Comparability exercise based on quality and toxicity data, as endorsed by HAs, confirmed that Phase 2 and Phase 3 quality were equivalent
  - *In vivo* study on Phase 3 product expression in mice muscles demonstrated comparable levels of expression in myofibers by ImmunoHistoChemistry

↳ **In vivo expression profile was not impacted by the manufacturing changes between Phase 2 lots and Phase 3 ones**

sanofi aventis
Because health matters

FGF1 positive myofibers including only muscle with needle injection site



- High variability in all groups
- Similar mean level on FGF1 positive myofibers

Phase 2 batch: Batch 50DNA97 in NaCl 150 mM

Phase 3 batch: Batch LG06005 in NacL 150 mM

Phase 3 batch: Batch LG06005 in NacL 150 mM + Tris 2.5 mM

sanofi aventis
Because health matters

Overall Conclusions on TAMARIS study

- The Phase 3 study, given its robust features, provides a reliable assessment of NV1FGF efficacy and safety at 1 year – long term safety follow-up ongoing
- Though IM administration of NV1FGF was proven in a Phase 1 study to lead to FGF-1 expression in patients' muscles, the study drug, at the tested dose of 4mg x 4 every two weeks, seems ineffective, as compared to placebo, in translating the FGF-1 protein expression into a meaningful and sustained clinical benefit for the CLI patients
- No formulation change nor treatment mix-up could explain the TAMARIS results
- The significant difference in the secondary endpoint observed in the small TALISMAN Phase 2 trial in favor of NV1FGF is probably a chance finding.
- TAMARIS results may question the concept of gene based therapeutic angiogenesis applied to CLI in its extrapolation from the impressive results seen in small animals into humans suffering from complex and chronic vascular disease.

sanofi aventis
Because health matters