Medical and Translational Research Day

Implementation of phase I studies in trauma surgery and neurosurgery in Luxembourg

Gerich, T.G., Centre Hospitalier, Luxembourg

Carrino, J., Hospital for Special Surgery, New York, USA

Bosen, M, Copenhagen University Hospital Bispebjerg and Frederiksberg, Denmark

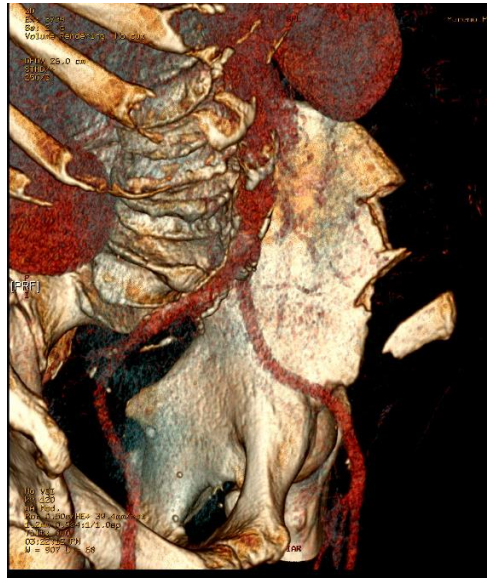
Frijns, D., Climsquare Healthcare Consulting, Herne, Belgien

Theys, N., Novadip Biosciences, Mont-Saint-Guibert, Belgien

Dufrane, D., Novadip Biosciences, Mont-Saint-Guibert, Belgien

Luxembourg, October 16th 2024

Autologous bone graft - ”gold standard” for bone healing

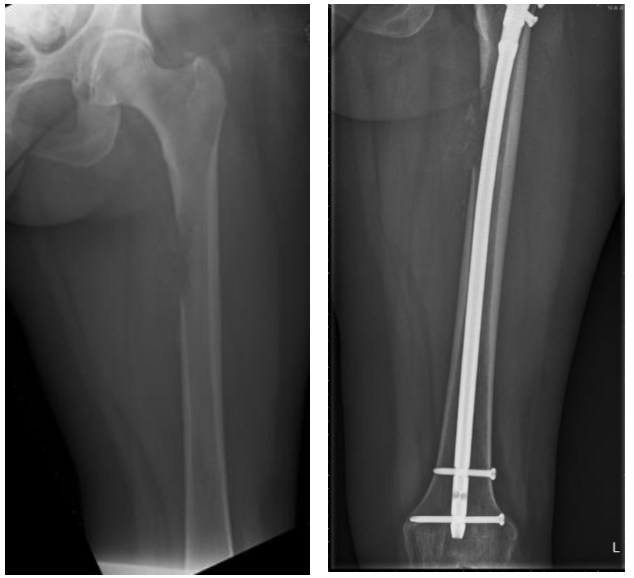


limited resources

- iliac crest, intramedullary canal of long bones

major complications at donor sites

- vascular injury
  - hematoma requiring surgical intervention
  - blood loss necessitating transfusion
- infections
- neurologic injury
- fractures



Main claim of all new bone substitutes

- Avoiding the autologous bone graft harvesting

NVD-003

Autologous, scaffold-free, stem cell-based transplant from adipose tissue

Osteogenesis through secretion of osteogenic and angiogenic growth factors

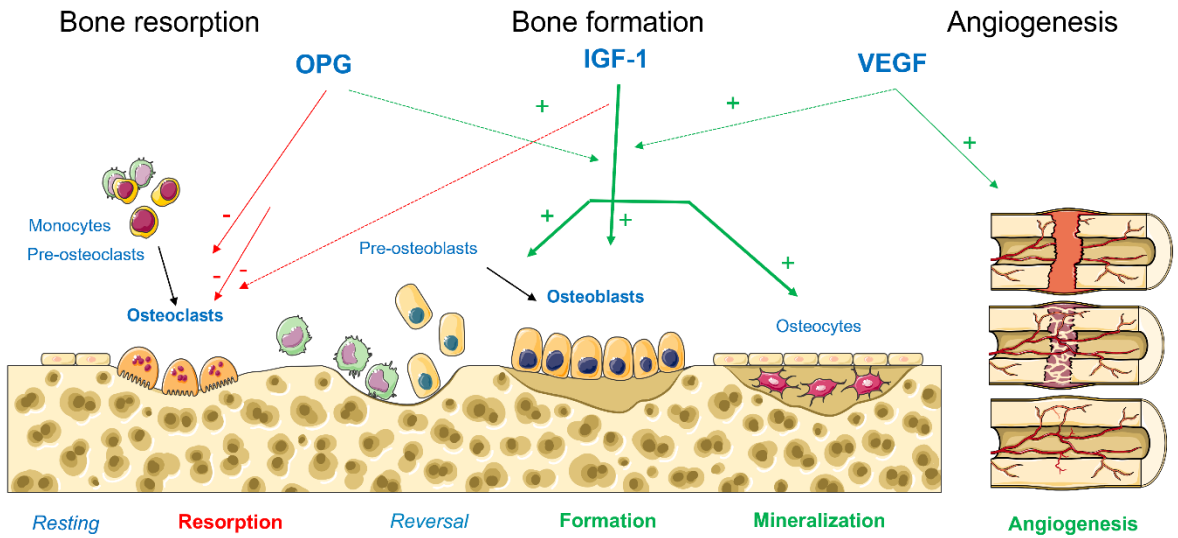
- Insulin Like Growth Factor-1 (IGF-1)
- Vascular Endothelial Growth Factor (VEGF)

reduction of osteoclast activity

- Osteoprotegerin (OPG)

Callus formation

- self-secreting extracellular matrix
- High mineral content (approx. 40 % Hydroxylapatit / Beta-Tricalciumphosphat)
- Facilitated adhesion and survival of osteogenic cells



Review > NPJ Regen Med. 2021 Mar 29;6(1):18. doi: 10.1038/s41536-021-00133-3.

Scaffold-free cell-based tissue engineering therapies: advances, shortfalls and forecast

Andrea De Pieri [1 2 3], Yury Rochev [2], Dimitrios I Zeugolis [4 5 6]

Affiliations + expand

PMID: 33782415 PMCID: PMC8007731 DOI: 10.1038/s41536-021-00133-3

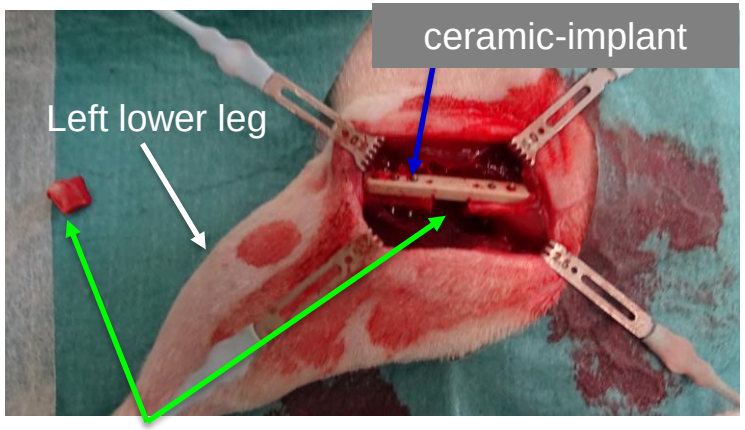
Observational Study > Medicine (Baltimore). 2015 Dec;94(50):e2220. doi: 10.1097/MD.0000000000002220.

Scaffold-free Three-dimensional Graft From Autologous Adipose-derived Stem Cells for Large Bone Defect Reconstruction: Clinical Proof of Concept

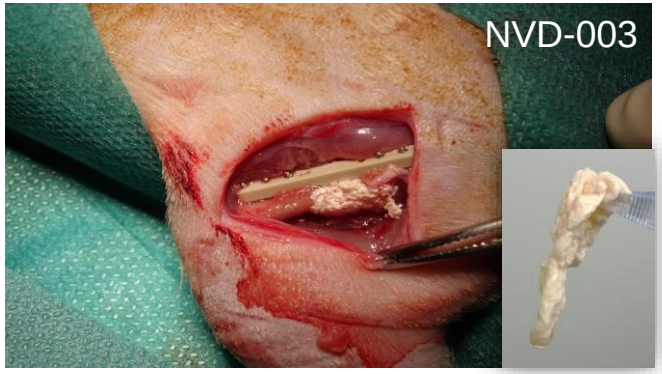
Denis Dufrane [1], Pierre-Louis Docquier, Christian Delloye, Hélène A Poirel, Wivine André, Najima Aouassar

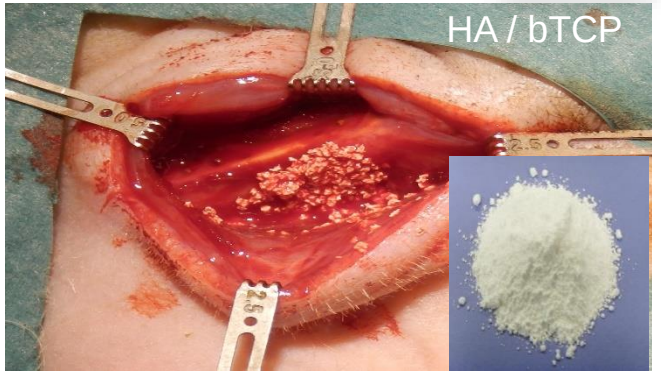
Critical size bone defect model - nude rat

Test
Item

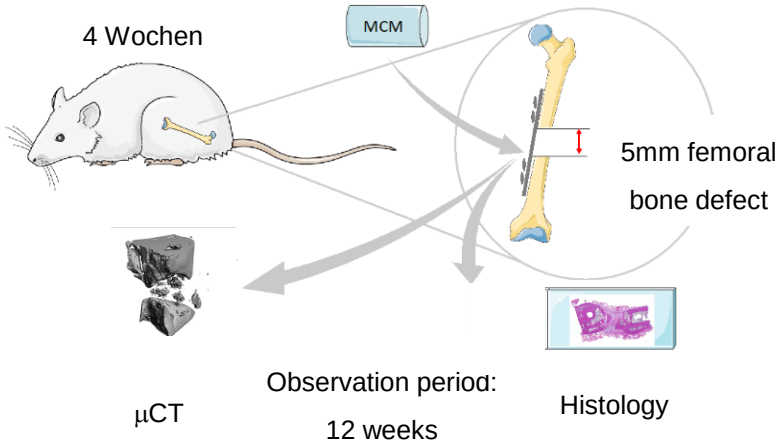


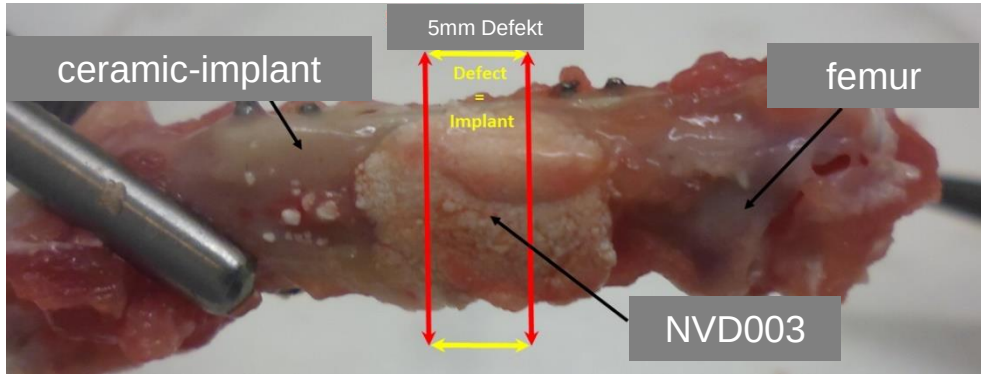
5mm Defekt





femur 4 weeks after implantation





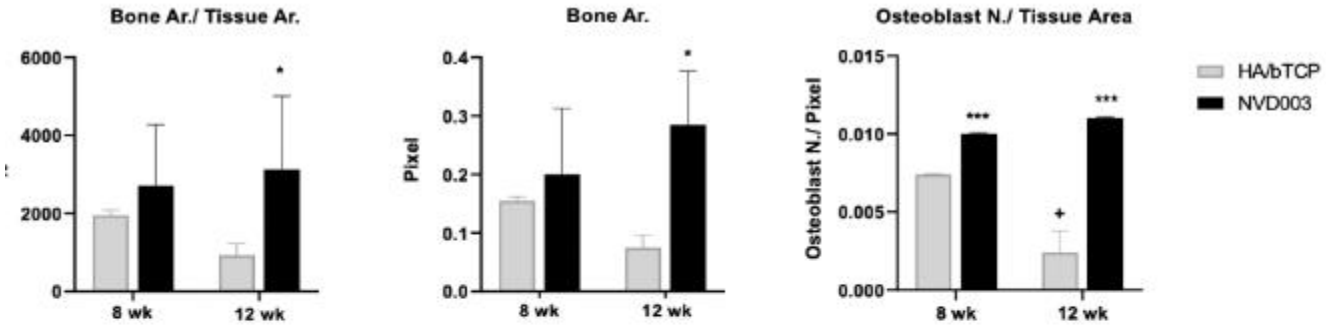
critical-size bone defect (CSBD) of 5 mm

left femur of immunodeficient nude male rats.

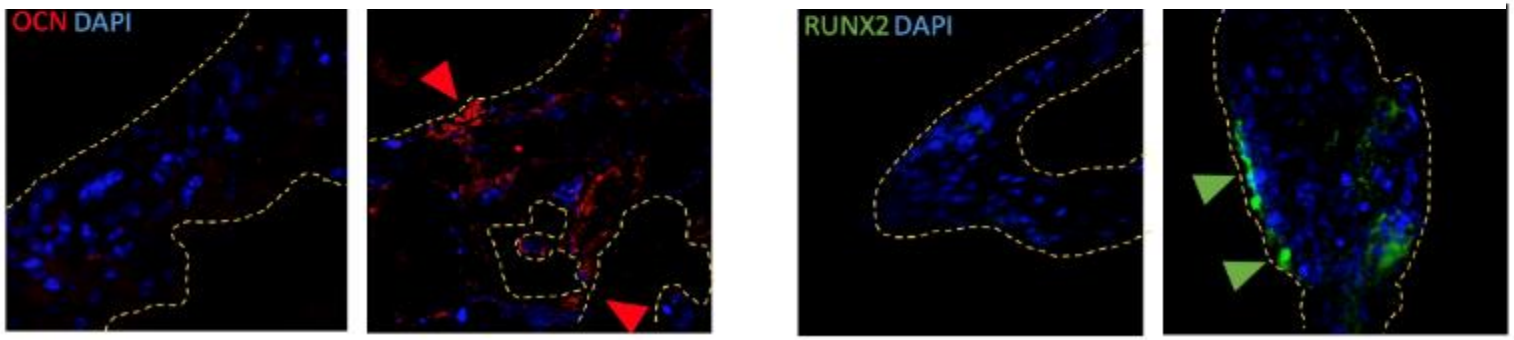
Four weeks after the bone defect, rats underwent

second surgery to implant NVD003 or HA/TCP.

NVD003 de novo bone formation
in a critical size bone defect



HA / bTCP NVD003 HA / bTCP NVD003



(OCN osteogenic cells) and

(RUNX2 transscription factor)

Demonstration of bone formation with NVD-003

inside the critical size bone defect.

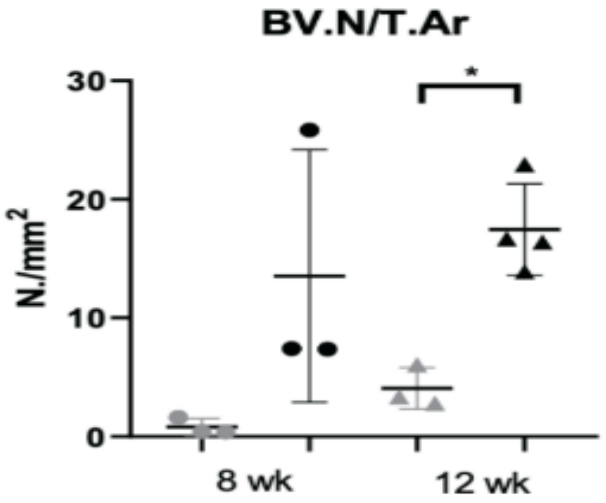
Hydroxyapatite alone did not elicit any bone

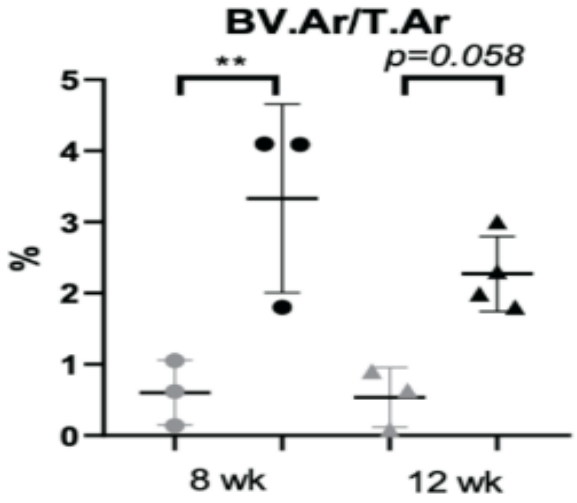
formation

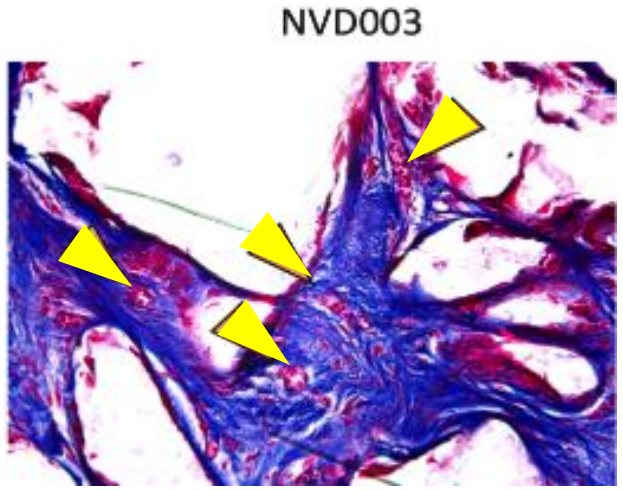
Performed by Prof A James, Department of Pathology

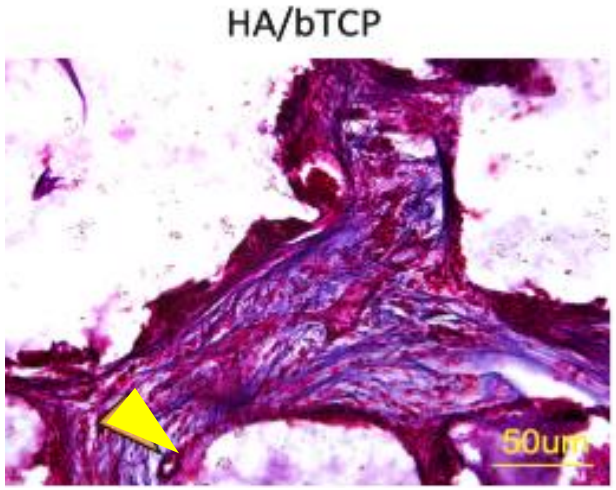
JOHNS HOPKINS UNIVERSITY

NVD003 – de novo vascularisation









significant **improvement of the revascularization** was found for NVD003 as determined by

- increase of blood vessel number (BV.N) and
- blood vessel area (BV.A)

in comparison to HA/TCP at 2- and 3-months post-implantation

Anfertigung Prof A James, Department of Pathology

JOHNS HOPKINS UNIVERSITY

Objective of this first study on humans

1. To evaluate the short and long-term safety of NVD-003 in patients with post-traumatic lower limb pseudarthrosis over a period of up to 24 months after implantation.
2. To evaluate the clinical and radiological efficacy of the NVD-003 graft for this indication.

EudraCT 2018-000299-13
approved by institutional ethics committees in Belgium, Luxembourg, and Switzerland

prospective, single-arm clinical study conducted in 5 centers across 2 countries:

Eligible patients were:

- ≥ 18 years old
- confirmed non-union of a

  single metaphyseal / diaphyseal bone defect in the lower extremity
- maximum size of 4 cm
- normal or low bone density (bone mineral density T-score > -2.5)
- no bisphosphates or other bone-modulating agents at the time of enrollment

NUSS (Non-Union Severity Score)

NUSS Parameter		PALU 101	PALU 102	PALU 103
Bone	Bone quality	Good	Good	Good
	Primary injury - open or closed fracture	Open grade I	Open grade IIIB and IIIC	Closed
	Number of previous interventions on the bone to procure healing	< 2	> 4	2 - 4
	Invasiveness of previous interventions	IM nailing	IM nailing	IM nailing
	Adequacy of primary surgery	Inadequate stability	Adequate stability	Adequate stability
	Weber & Cech group	Oligotrophic	Oligotrophic	Hypertrophic
	Bone alignment	Anatomical alignment	Anatomical alignment	Anatomical alignment
	Bone defect-gap	> 3cm	0.5 - 1cm	> 3cm
Soft Tissue	Soft tissue status	Minor scarring	Previous free flap	Minor scarring
Patient	ASA grade	1 or 2	1 or 2	1 or 2
	Diabetes	No	No	No
	Blood tests: FBC: WCC > 12	No	No	No
	Blood tests: ESR > 20	No	Yes	No
	Blood tests: CRP > 20	No	No	No
	Clinical infection status	Clean	Clean	Previously infected
	Drugs: Steroids	No	No	No
	Drugs: NSAIDs	Yes	Yes	No
	Smoking	No	Yes	No
Total NUSS		**16**	**29**	**15**

Review > Injury. 2008 Sep:39 Suppl 2:S59-63. doi: 10.1016/S0020-1383(08)70016-0.

Classification of non-union: need for a new scoring system?

Giorgio Maria Calori [1], Mark Phillips, Sharanpal Jeetle, Lorenzo Tagliabue, P V Giannoudis

NVD003 - production and characterisation

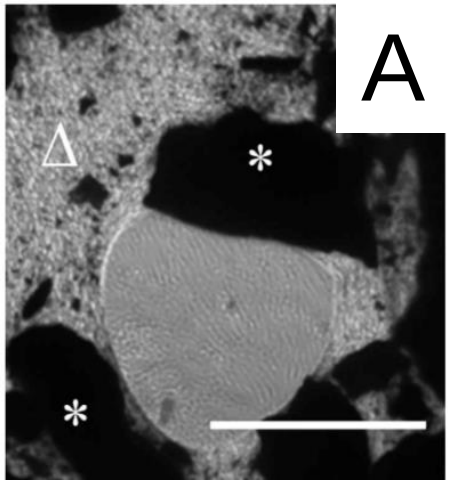
Procurement of mesenchymal stem cells

- Minimally invasive liposuction
- Isolation of mesenchymal stem cells and ex vivo expansion

Differentiation in osteogenic medium and addition of HA / TCP

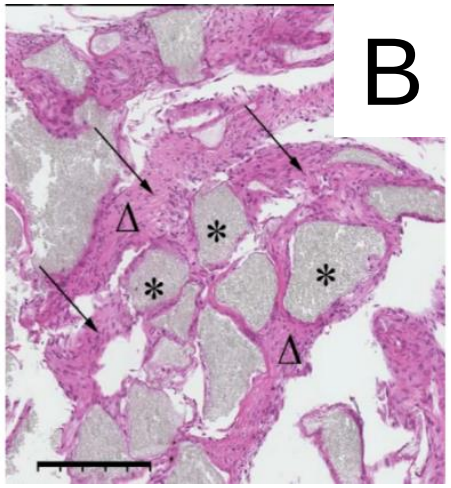
- Secretion of an extracellular matrix and formation of osteogenic material
- Scaffold-free, 3-dimensional osteogenic implantable substrate (approx. 8 weeks)

Quality control of NVD-003



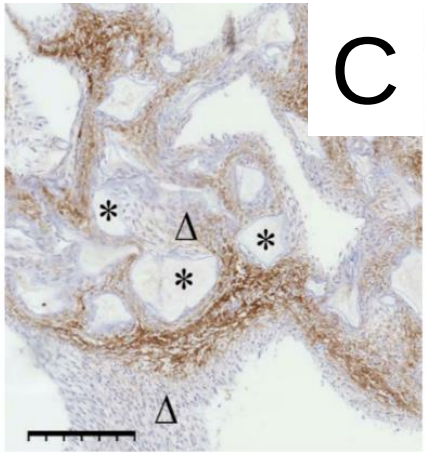
Phase contrast microscopy

3-7 days after the addition of HA/TCP particles showing integration into the extracellular matrix secreted by the cells (bar = 1000 μm)



Hematoxylin-eosin staining

8 weeks after the addition of HA / TCP particles (original magnification x10; bar = 250 μm)



Osteocalcin staining

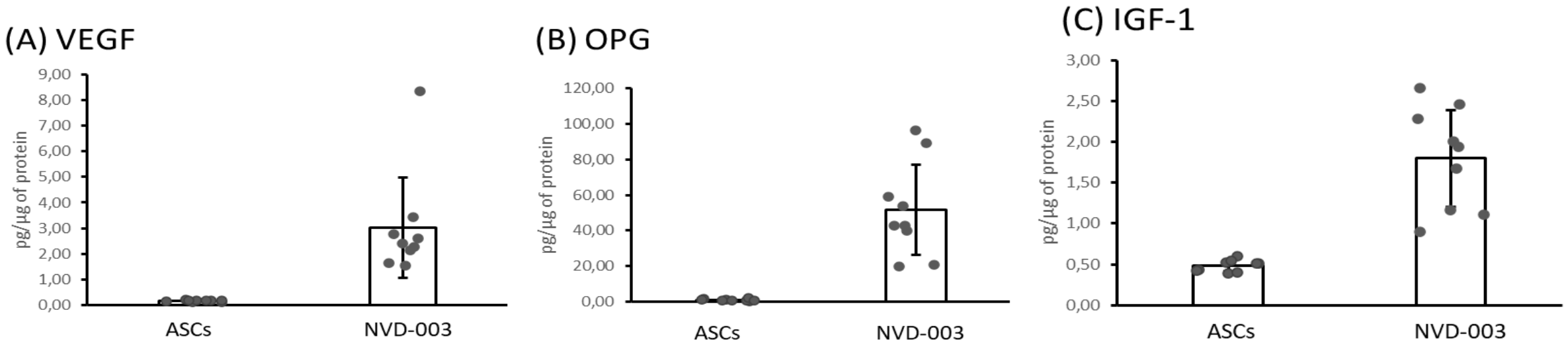
8 weeks after the addition of HA / TCP particles (original magnification x10; bar = 250 μm)

Macroscopic view

of ready-to-implant NVD.003 (8 weeks after addition of HA/TCP) showing scaffold-free three-dimensional structure

*	HA / TCP particles
Δ	extracellular matrix
→	adipose-derived cells

Protein content in naïve adipose-tissue-derived mesenchymal stem cells and NVD-003



Data represent the mean and standard error of the mean for 9 observations per group.

VEGF vascular endothelial growth factor.

OPG osteoprotegerin

IGF-1 insulin-like growth factor 1

Bone reconstructive surgery

Approximately 12 weeks after the liposuction procedure

standard-of-care, non-restricted bone reconstructive surgery

using NVD-003 to fill the bone void.

NVD-003 was used as a single agent

- no adjunctive autologous bone grafts (e.g., iliac crest grafting)
- no bone-enhancing agents (e.g., rhBMP-2)

For one patient, a preparatory reconstructive Masquelet technique was performed at the moment of the adipose tissue collection.

Terminology	Safety Population	(Study participants who have signed consent)
	Efficacy Population	(Participants who have received NVD003)

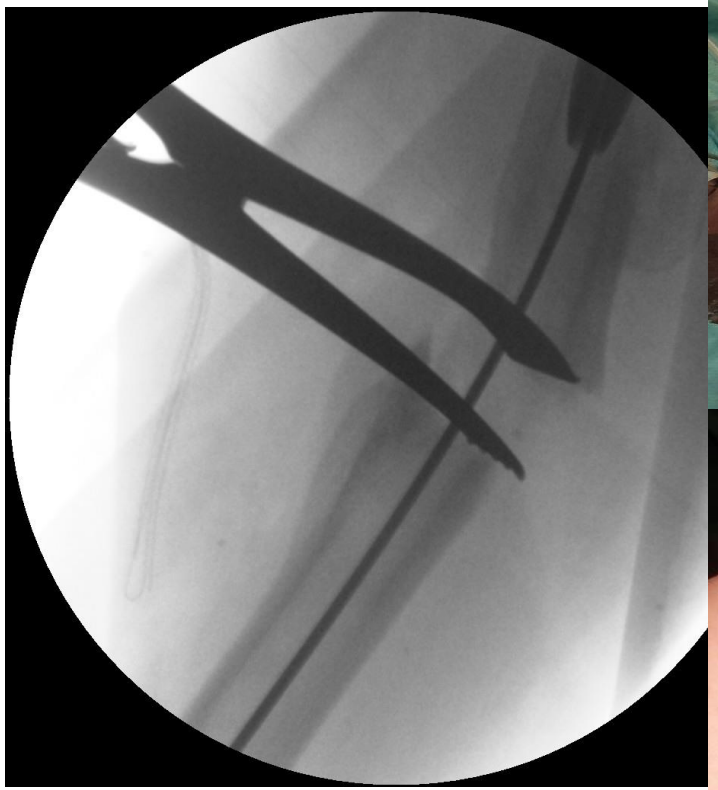
Participants

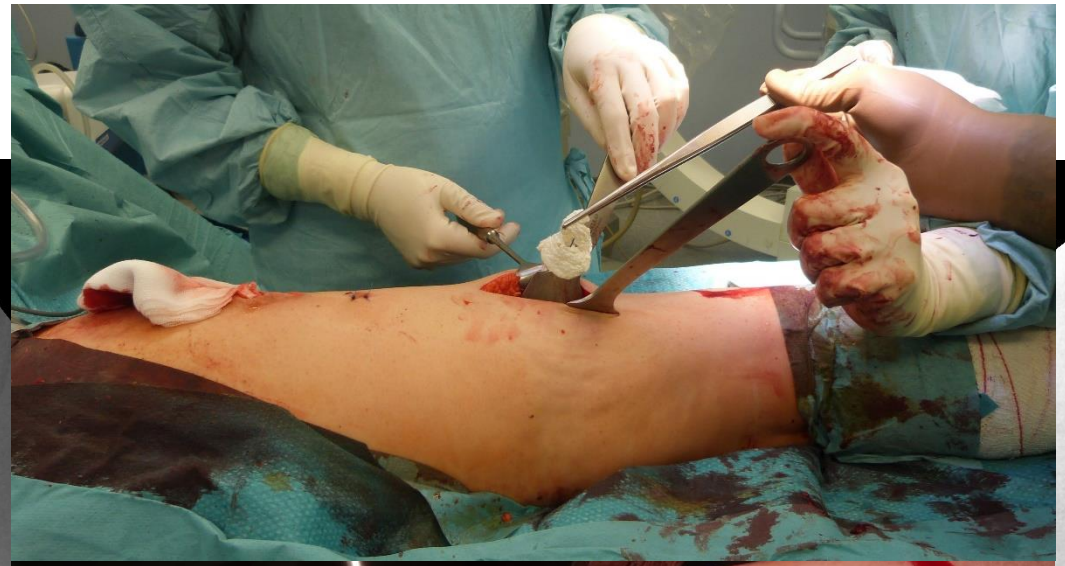
Participants	4 men, 5 women	
Age	median 56 years	(21-74)
Target bone defects	4 tibial and 5 femoral traumatic fractures	
primary fracture characteristics and treatment history:		
	High heterogeneity, up to 14 previous corrective surgical interventions	
bone defect size	0.5-1 cm	5 participants (55.6%)
	1-3 cm	1 participant (11.1%)
	3 cm	3 participants (33.3%)
mean defect volume	9.6 ± 6.6 cm^3	(median volume, 9 [2.5-21.5] cm^3)
time since the primary fracture at the origin of non-union:		
	94.74 ± 150.27 months	(median, 25.3 (14.4-473.7) months
American Society of Anesthesiologists status		grade 1 or 2 (healthy or mild systemic disease)
		no diabetes, 3 current smokers

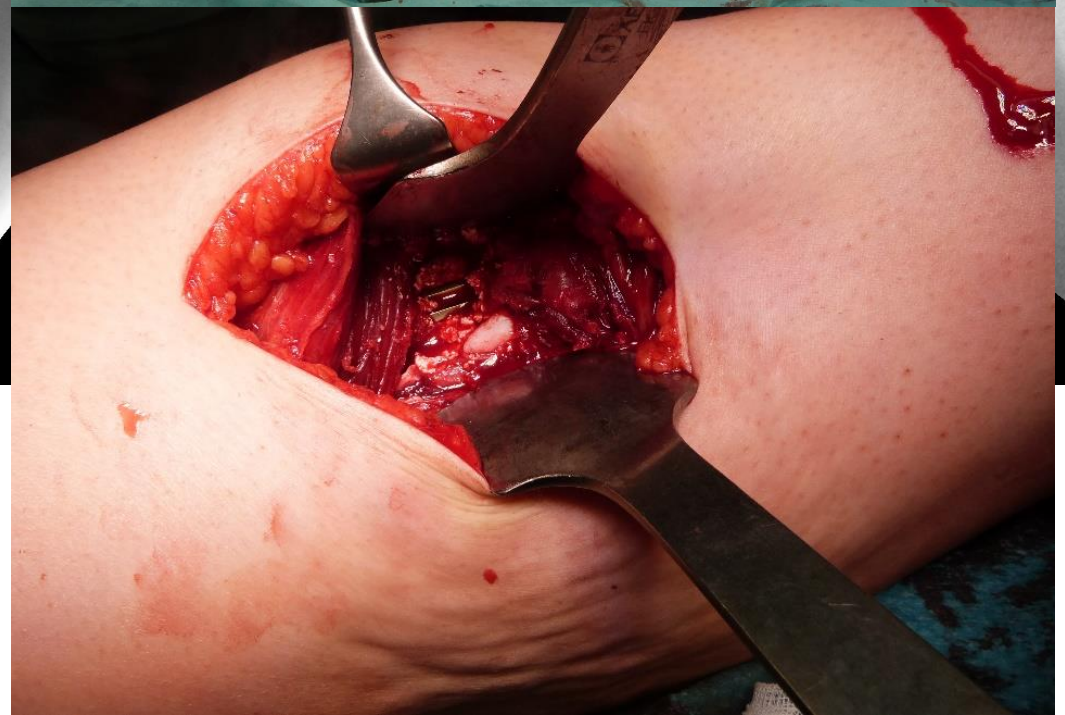
Demographics and characteristics of individual participants in the efficacy population

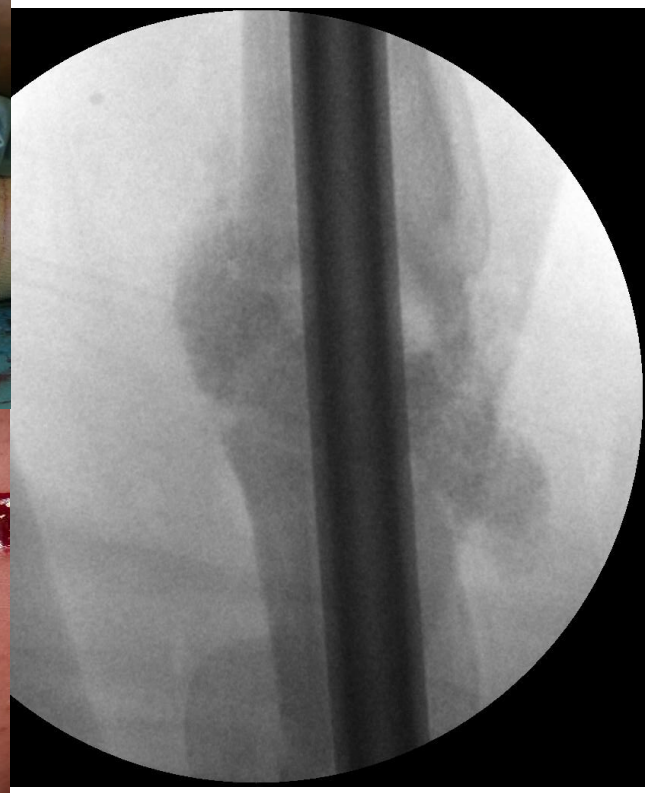
Participant	P1 (PABE102)	P2 (PABE301)	P3 (PABE401)	P4 (PABE402)	P5 (PABE501)	P6 (PABE504)	P7 PALU101)	P8 (PALU102)	P9 (PALU103)
Sex	Female	Female	Female	Female	Male	Male	Female	Male	Male
Age (years)	57	73	74	56	46	62	21	44	38
Weight (kg)	58	72	92	64	88	79	51	82	92
Smoking status	Yes	No	No	Yes	No	No	No	Yes	No
Primary fracture date (month year)	Apr 2017	Oct 2016	Sep 2018	Nov 2018	Nov 2014	Jun 2016	Aug 2016	Jul 2013	Jan 2006
previous interventions (n)	1	1	3	4	8	6	1	18	4
fracture location	Femur (left)	Femur (right)	Tibia/fibula (right)	Tibia/fibula (right)	Femur (right)	Tibia (left)	Femur (right)	Tibia/fibula (right)	Femur (left)
Fracture type	closed high velocity (triple) fracture	closed low velocity fracture	closed low velocity fracture (including pelvic fracture)	closed low velocity fracture	grade IIIB/IIIC high velocity fracture	closed high velocity fracture	open grade I high velocity fracture	grade IIIB/IIC high velocity fracture	closed high velocity fracture
Height/size of fracture defect (cm)	0.5–1	0.5–1	2–3	> 3	0.5–1	0.5–1	> 3	0.5 – 1	> 3
NUSS Score	18	9	16	22	21	13	16	29	15
Time since primary fracture to GS (month)	20.3	27.1	17.8	18.2	51.4	42.5	29.4	71.9	171.1
Implant volume (cc)	13.7	9.3	16.5	16.8	11.4	15.8	17.9	16.6	17.2

NVD003 (PALU 101)



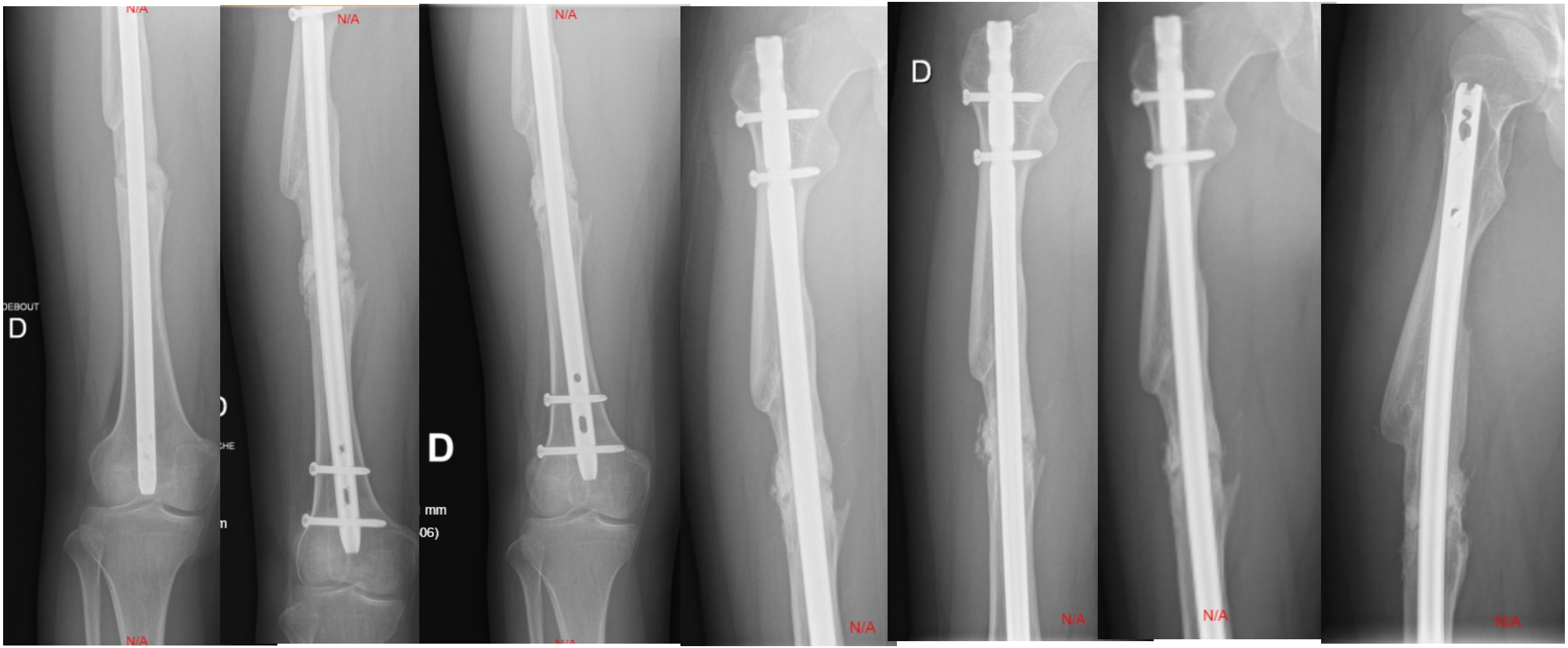






NVD003 (PALU 101)

PRE-op V3 V5 V6 Vadd 2 Vadd3 V7



Extended Lane and Sandhu radiological scoring tool

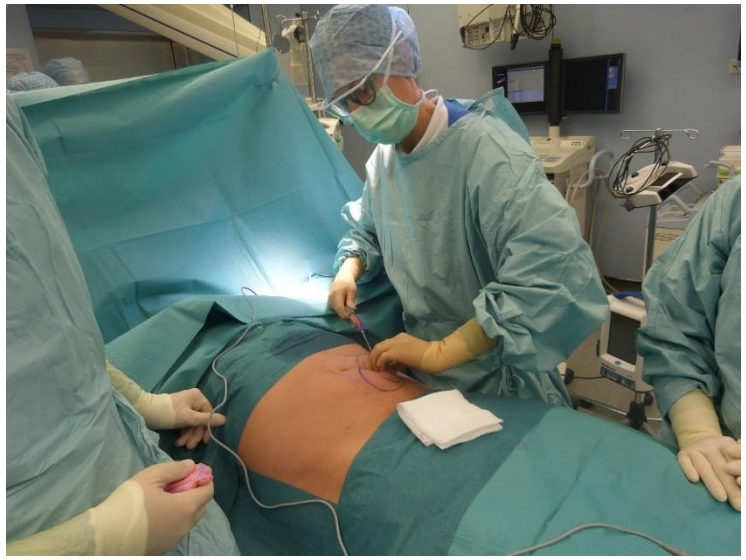
Bone Formation (filling of the longitudinal gap against baseline)	**Bone Union** (filling of the transverse gap against baseline)	**Bone Remodeling**
0 – Complete longitudinal defect	0 – Complete transverse defect	0 – No evidence of remodeling against the baseline
1 – Up to 25%	1 – Up to 25%	1 – Possible remodeling of intramedullary canal
2 – Up to 50%	2 – Up to 50%	2 – Full remodeling of intramedullary canal and cortex
3 – Up to 75%	3 – Up to 75%	
4 – Up to 100%	4 – Up to 100%	
5 – 100%	5 – 100%	
Maximum score = 5	Maximum score = 5	Maximum score = 2
Total extended LSS = sum scores for bone formation, bone union and bone remodeling **(maximum = 12)**		

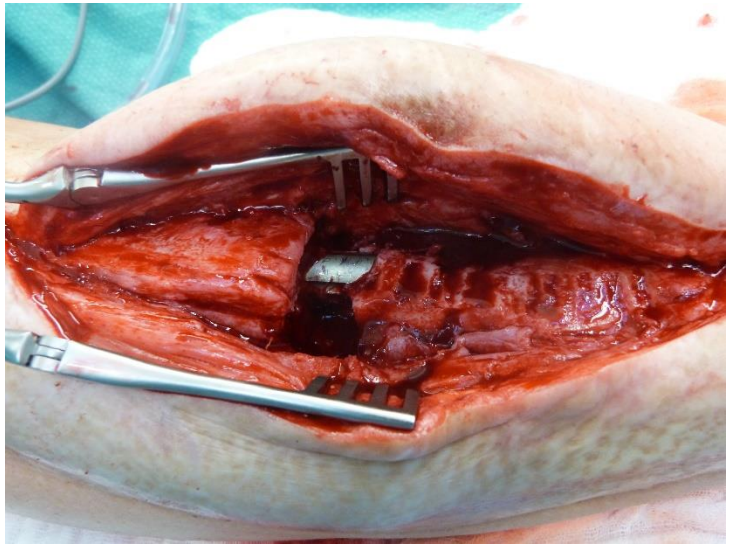
Orthop Clin North Am. 1987 Apr;18(2):213-25.

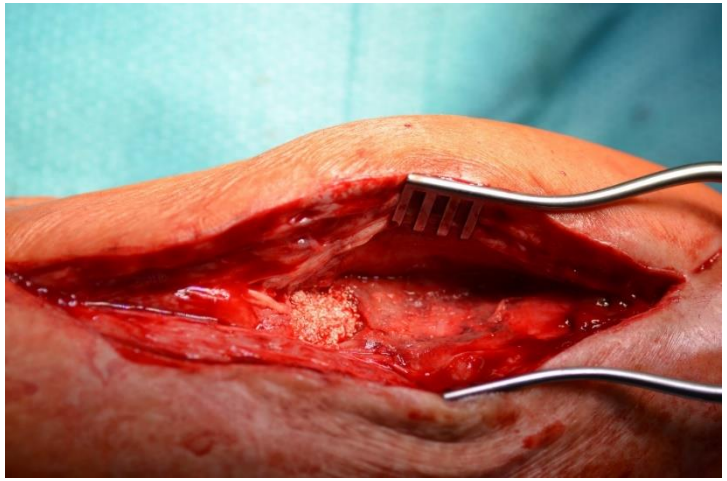
Current approaches to experimental bone grafting

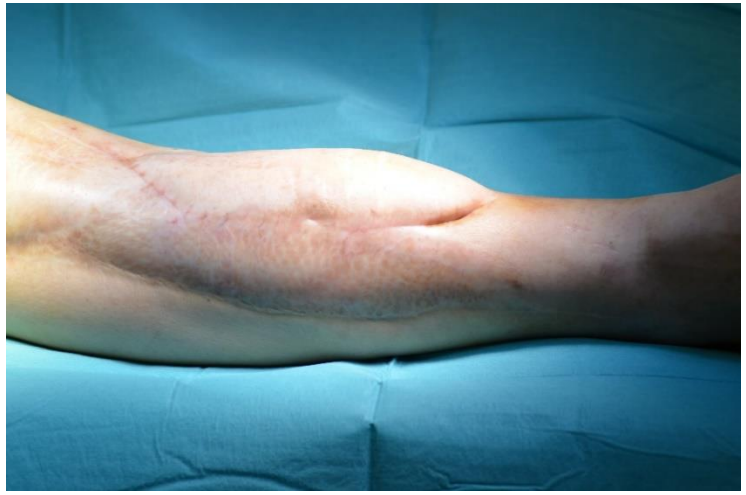
J M Lane, H S Sandhu

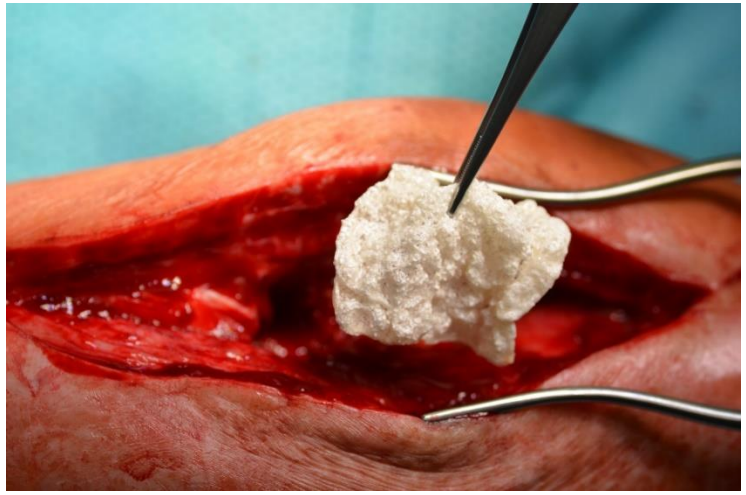
NVD003 (PALU 102)

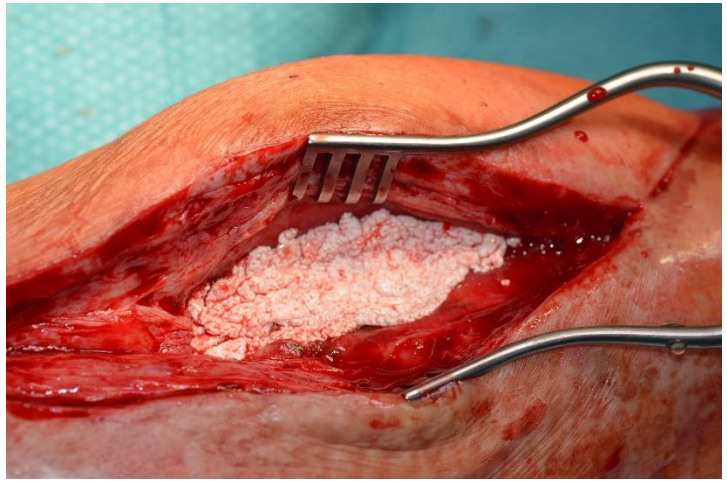






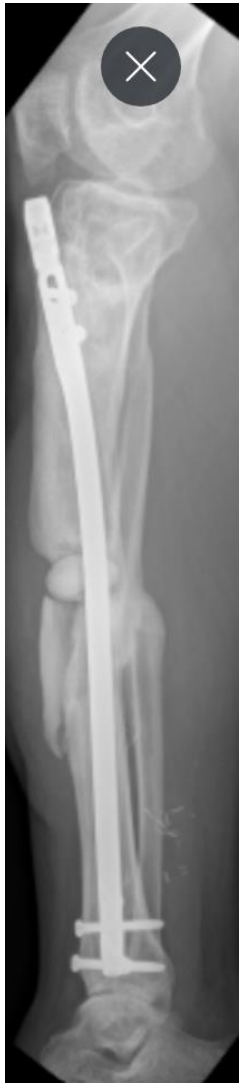


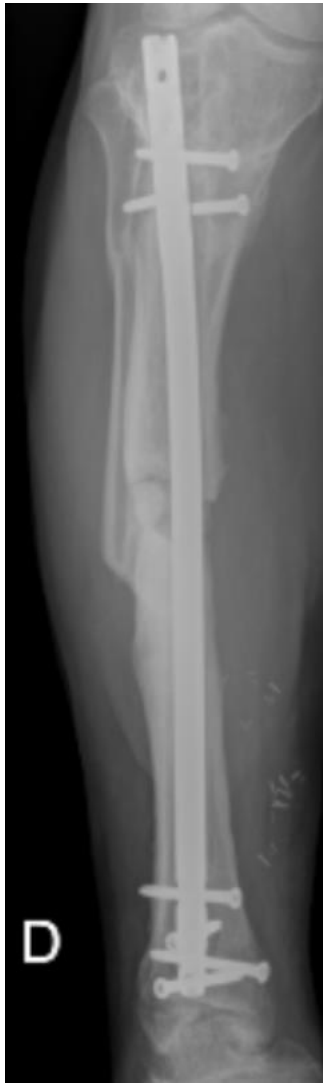


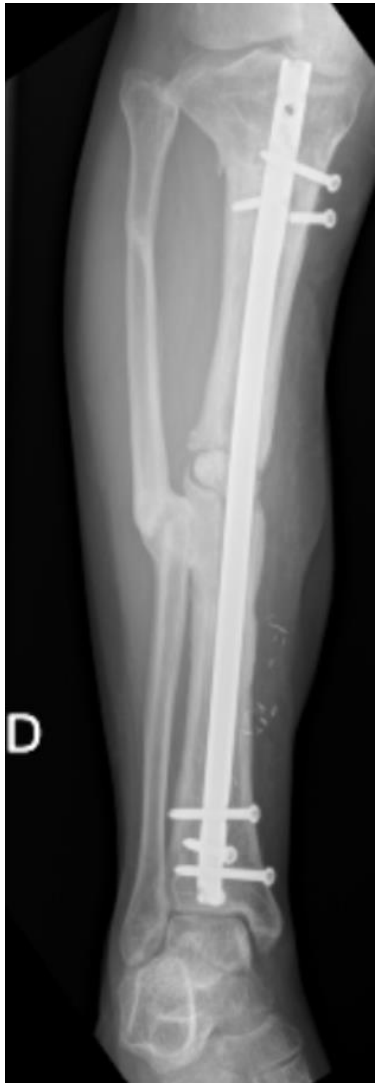


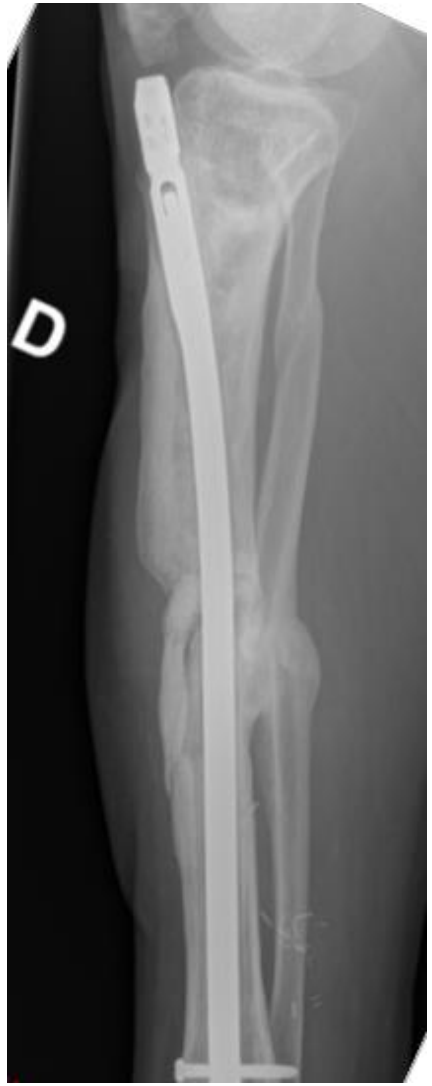
NVD003 (PALU 102)

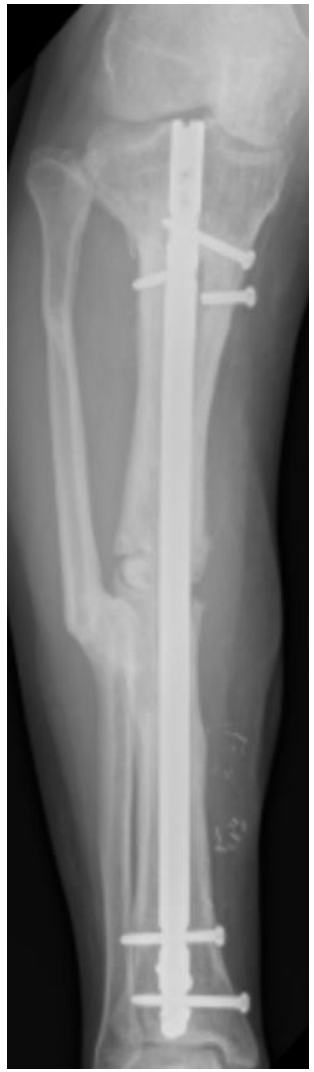
PRE-op V3 V5 V6 Vadd1



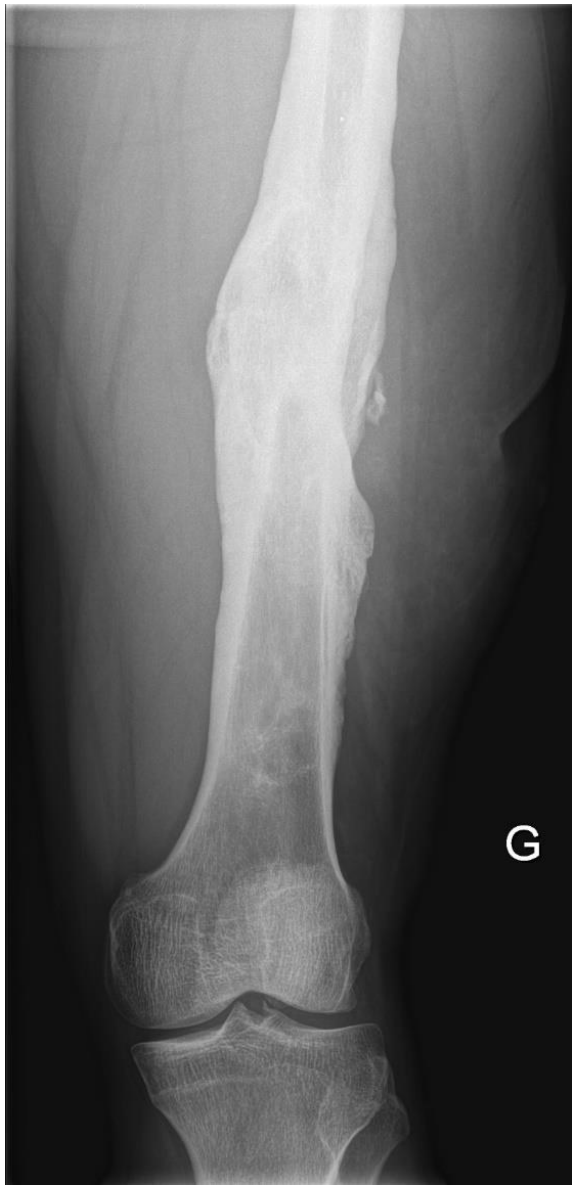




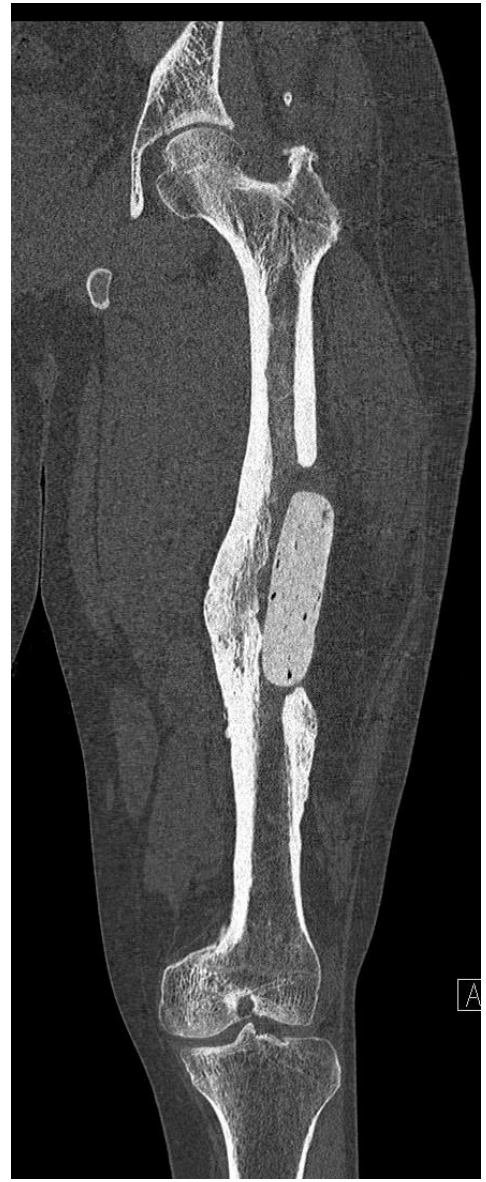




NVD003 (PALU 103)

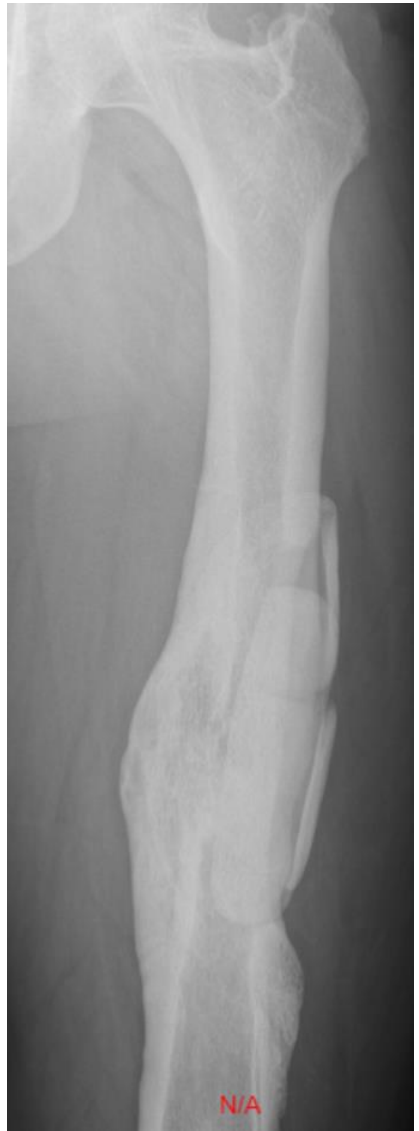


Date	Dossier	Prescripteur
PENICILLINE G	.	R
AMPICILLINE	R	.
AMOX/AC-CLAV	R	.
CEFAZOLINE	.	R
CEFUROXIME	R	.
CEFTRIAXONE	S	.
MINOCYCLINE	.	S
CEFTAZIDIME	S	.
CLINDAMYCINE	.	R
ERYTHROMYCINE	.	R
VANCOMYCINE	.	S
TRIMETH /SULFAMETHOX	S	.
AZTREONAM	S	.
PIP/TAZOBACTAM	S	.
GENTAMICINE	S	R
AMIKACINE	S	.
MUPIROCINE	.	S
CIPROFLOXACINE	R	.

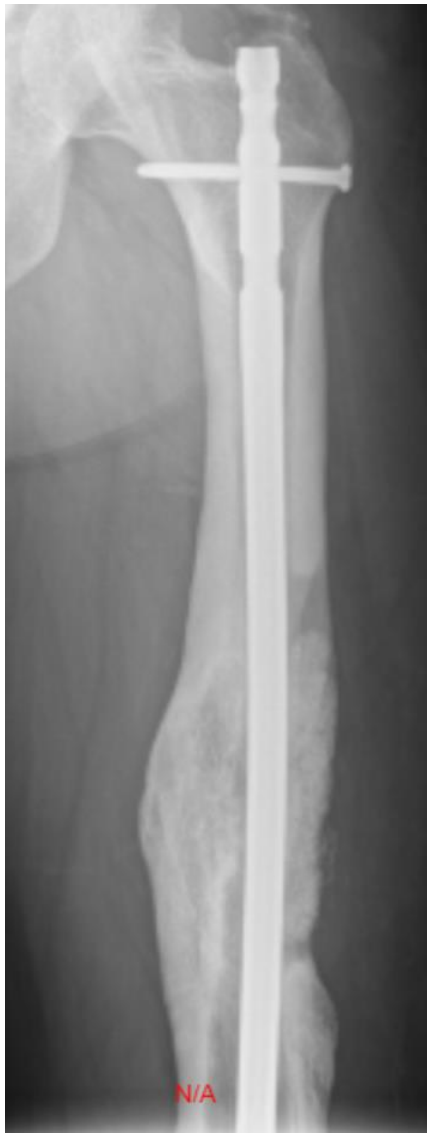


NVD003 (PALU 103)

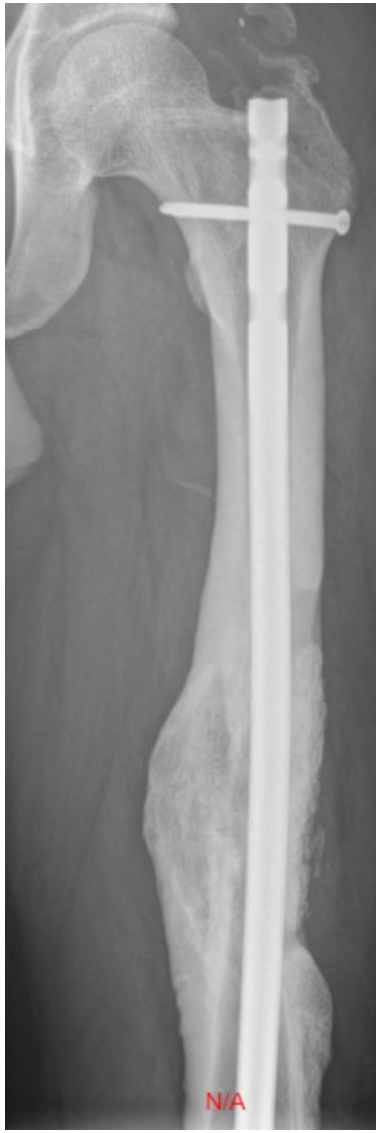
preoperative



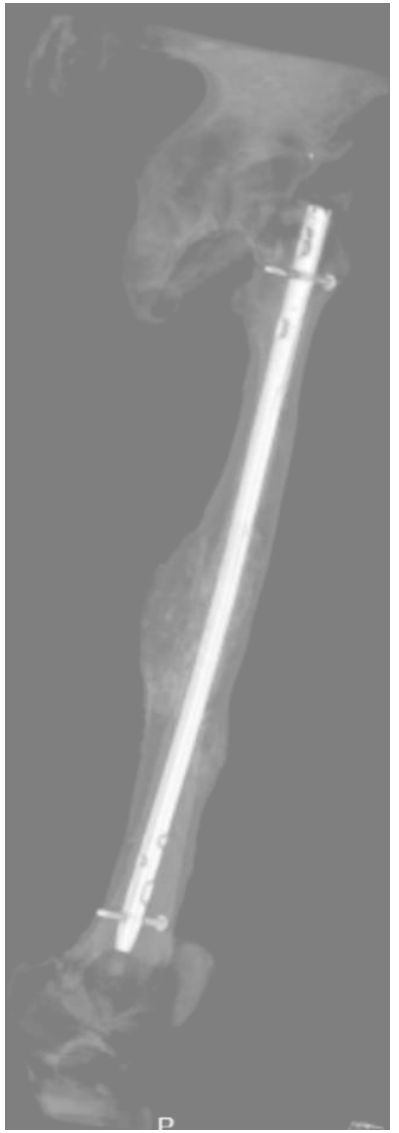
V3



V4

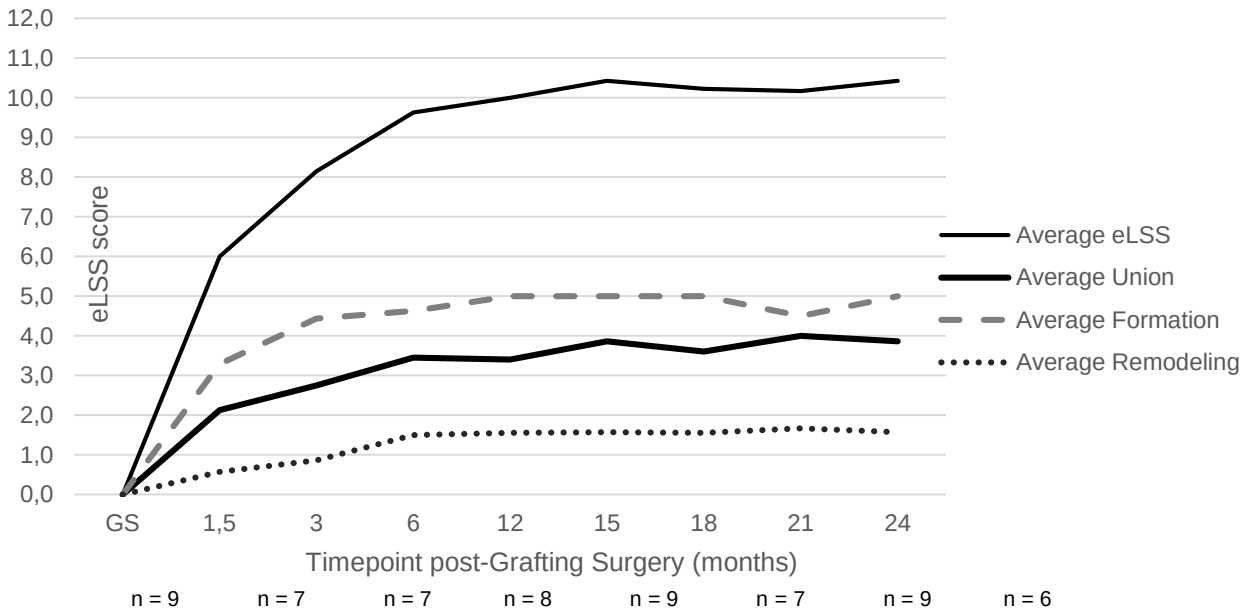


V5 (6months)



Evolution of average extended Lane and Sandhu scores

X-ray



CT

› Orthop Clin North Am. 1987 Apr;18(2):213-25.

Current approaches to experimental bone grafting

J M Lane, H S Sandhu

NVD003 EU Clinical Trial – Evolution of Clinical Healing

- 8 / 9 (89%) achieved clinical healing during the two years period of follow-up postoperative.
- median and mean time to clinical healing were 6 months and 9 months, respectively.
- 100% of patients with total weight bearing at 6 months and
- 7 / 9 (78%) normal walking at 2 years

Clinical healing	Week 6	Month 3	Month 6	Year 1	Month 15	Month 18	Month 21	Year 2
PABE102	No	Yes	Yes	Yes		Yes	Yes	Yes
PABE301	No	Yes	Yes	Yes	Yes	Yes		
PABE401	No	No	No	No	No	No	No	No
PABE402	No	No	No	Yes	No	No	Yes	Yes
PABE501	No	No	No	Yes		Yes		
PABE504	No	.	Yes	Yes	Yes	Yes	Yes	Yes
PALU101	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
PALU102	No	No	No	No	No	Yes		Yes
PALU103	No	Yes	Yes	Yes	Yes			Yes

Time to clinical healing
estimated by Kaplan-Meier survival analysis

Mean (Estimation is limited to the largest survival time if it is censored / Die Schätzung ist auf die größte Überlebenszeit beschränkt, wenn diese zensiert ist.)				Median			
Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound			Lower Bound	Upper Bound
9.2	2.5	4.3	14	**6 months**	4.5	0.0	14.8

Adverse Events considered related to the procedure in the efficacy population (MedDRA System Organ Class and Preferred Term)

		No. events	% of events	No. participants	% of participants
General disorders and administration site conditions	Edema peripheral	1	5.6	1	11.1
Injury, poisoning and procedural complications	Anemia postoperative	2	11.1	2	22.2
	Inflammation of wound	1	5.6	1	11.1
	Post procedural edema	1	5.6	1	11.1
	Postoperative wound complication	1	5.6	1	11.1
	Procedural nausea	1	5.6	1	11.1
	Procedural pain	5	27.8	4	44.4
Investigations	Alanine aminotransferase increased	1	5.6	1	11.1
	Aspartate aminotransferase increased	1	5.6	1	11.1
	C-reactive protein increased	1	5.6	1	11.1
	Gamma-glutamyl transferase increased	1	5.6	1	11.1
Musculoskeletal and connective tissue disorders	Arthralgia	1	5.6	1	11.1
Vascular disorders	Hypotension	1	5.6	1	11.1
Total		**19**	**100.0**	**5**	**55.6**

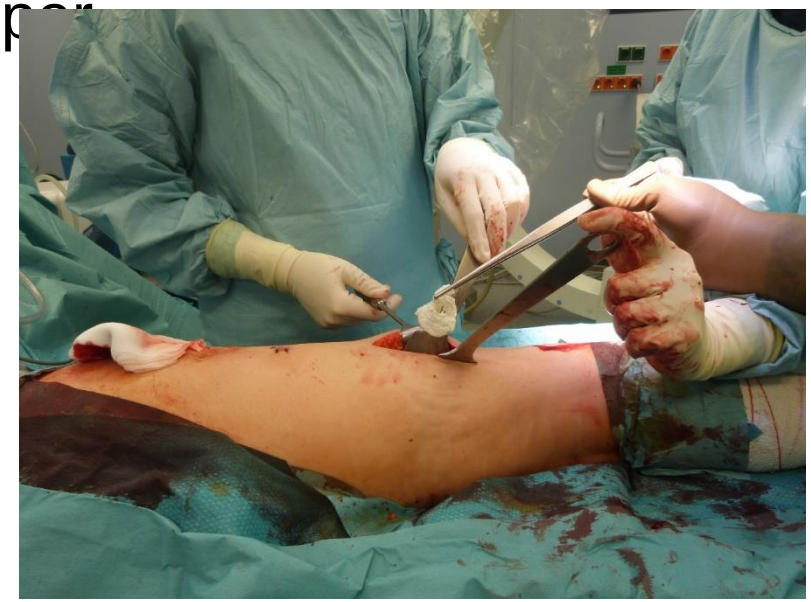
Adverse Event until 05.2022

Patient number	(S)AE description	(S)AE Seriousness	(S)AE start date	(S)AE stop date	(S)AE Intensity	(S)AE Action taken	(S)AE Outcome	(S)AE Relationship to procedure	(S)AE relationship to study drug
PALU101	anemia	No	20/09/2019	16/01/2020	Mild	Medication	Recovered/ Resolved	Unrelated to the procedure	Unrelated
PALU101	urinary tract infection	No	20/09/2019	16/01/2020	Mild	Medication	Recovered/ Resolved	Unrelated to the procedure	Unrelated
PALU101	major bronchial secretion	No	20/09/2019	18/11/2019	Mild	Medication	Recovered/ Resolved	Unrelated to the procedure	Unrelated
PALU102	left disc herniation L5-S1	No	28/12/2019	11/02/2020	Severe	Medication - Therapy	Recovered/ Resolved	Unrelated to the procedure	Unrelated
PALU102	Aggravated left disc herniation L5-S1.	Yes	04/02/2020	11/02/2020	Severe	Other drugs started - Other therapeutic measures	Recovered/ Resolved	Unrelated to the procedure	Unrelated
PALU103	post-operative anemia	No	20/04/2020	25/05/2020	Mild	Medication	Recovered/ Resolved	Definitely related to the procedure	Unrelated
PALU103	post-operative pain	No	15/04/2020	15/05/2020	Mild	Medication	Recovered/ Resolved	Definitely related to the procedure	Unrelated
PALU103	hypotension	No	08/04/2021	08/07/2021	Mild		Recovered/ Resolved	Unrelated to the procedure	Unrelated

Summary

NVD-003 implantation

- was not causally implicated in any of the recorded AEs
- did not produce any foreign body responses
- did not cause any unexpected delayed adverse events p
  long-term follow-up data available to date
- can promote healing in a critical size bone defect at 36
  months follow-up



NVDX3

LIH

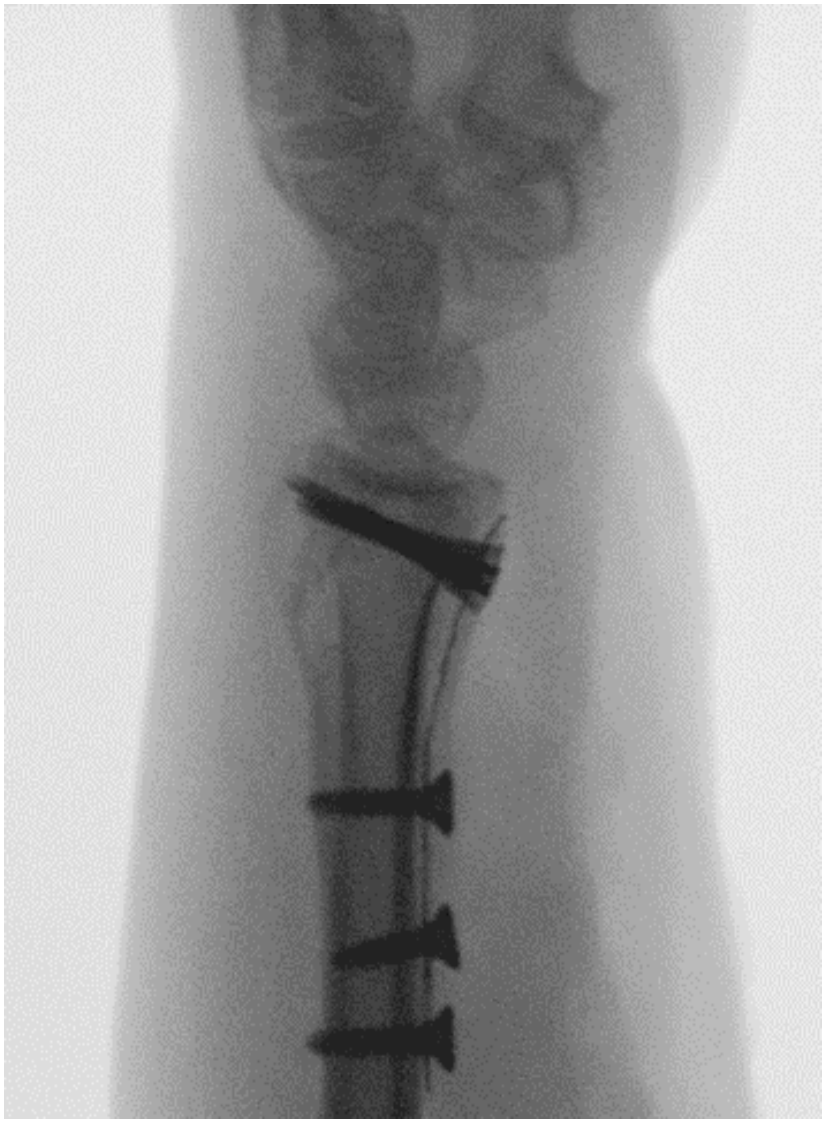
Study Rationale & Purpose

- **DRF – Distal Radius Fracture**
- ..often referred to as “wrist fractures”

✓ Chosen by Novadip as representative model for orthopedic and orthotopic indications

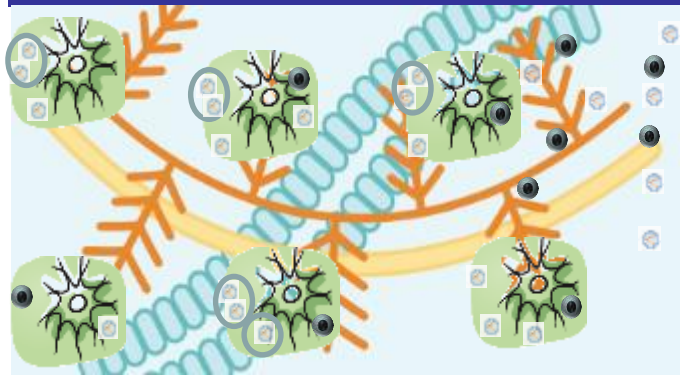
✓ Metaphyseal comminution DRFs are underappreciated injuries and are a common cause of chronic wrist pain and limited range of motion, negatively impacting the quality of life of the patients.

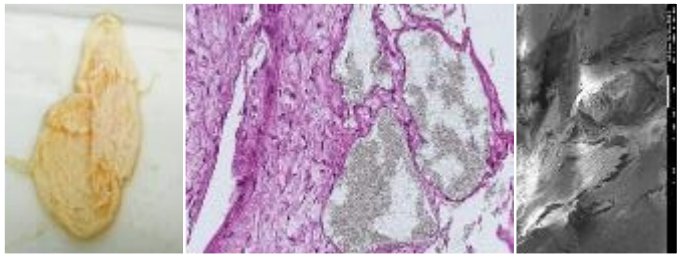
✓ Currently requires autologous bone harvesting and secondary iliac crest surgery or use of non-biologically active products / scaffolds



Off-the-Shelf product is derived from the autologous product

Autologous Product

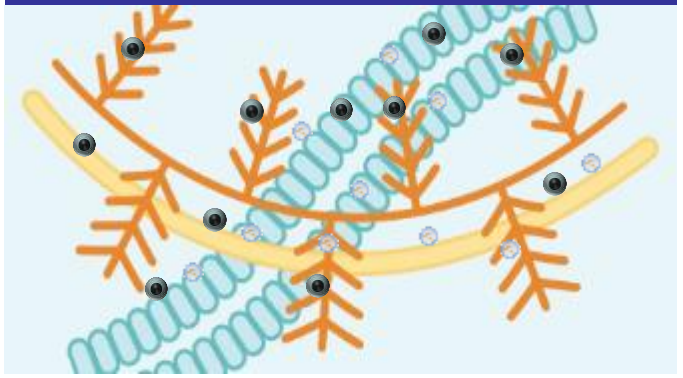


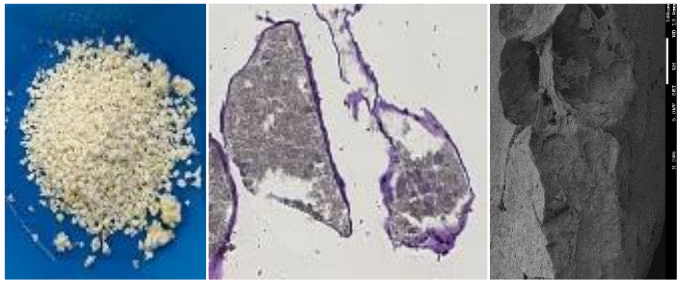


3D scaffold free autologous products is the basis of 3M³ Delivery platform

Lyophilization + sterilization

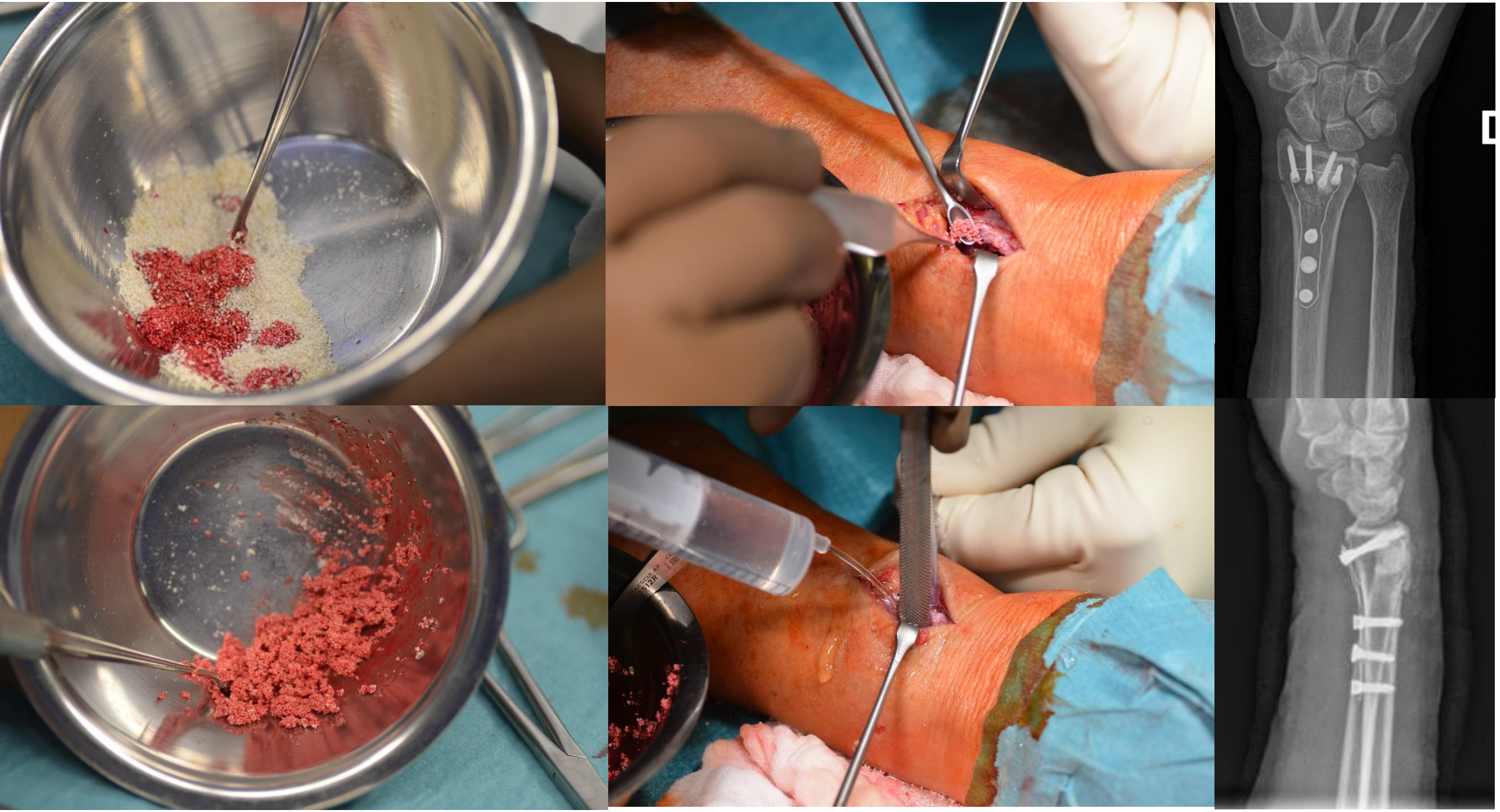
Off-the-Shelf Matrix





miRNA and growth factors enclosed in ECM

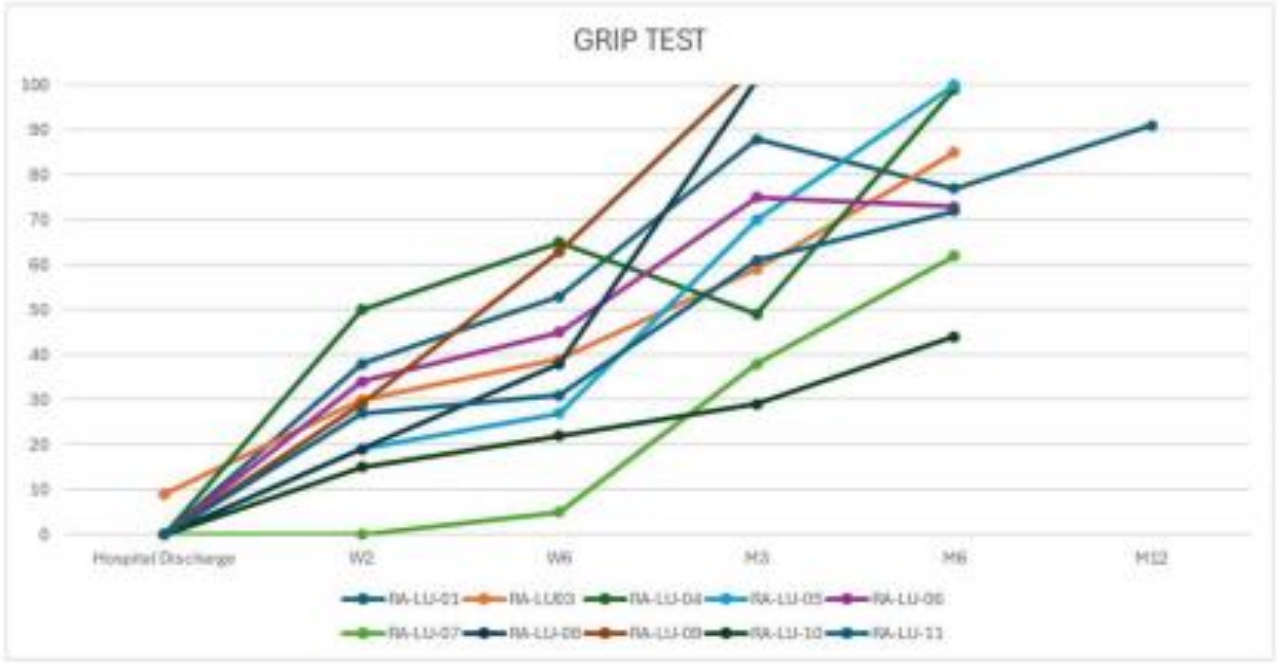
eLSS components



NVDX3 – Radius 1st Efficacy data

GRIP strength test

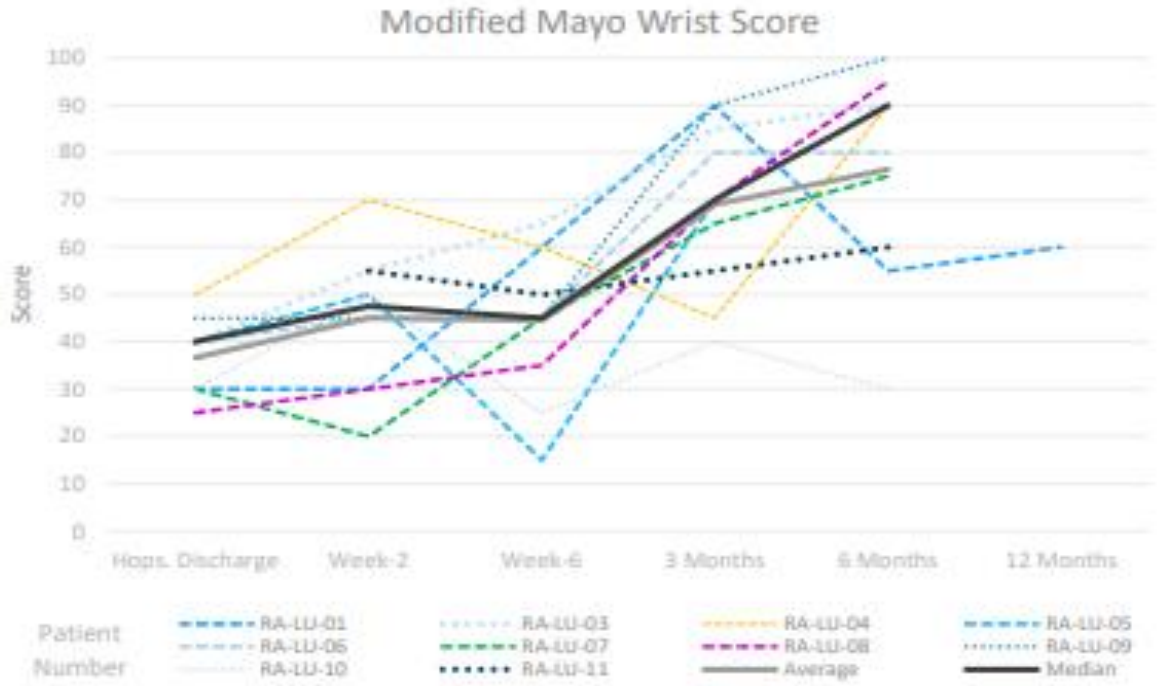
Values are expressed as the percentage of the strength on the contralateral (uninjured) side.



Improvement in the grip > 80% from Hospital Discharge (baseline) to the 6th month visit (10/10 patients)

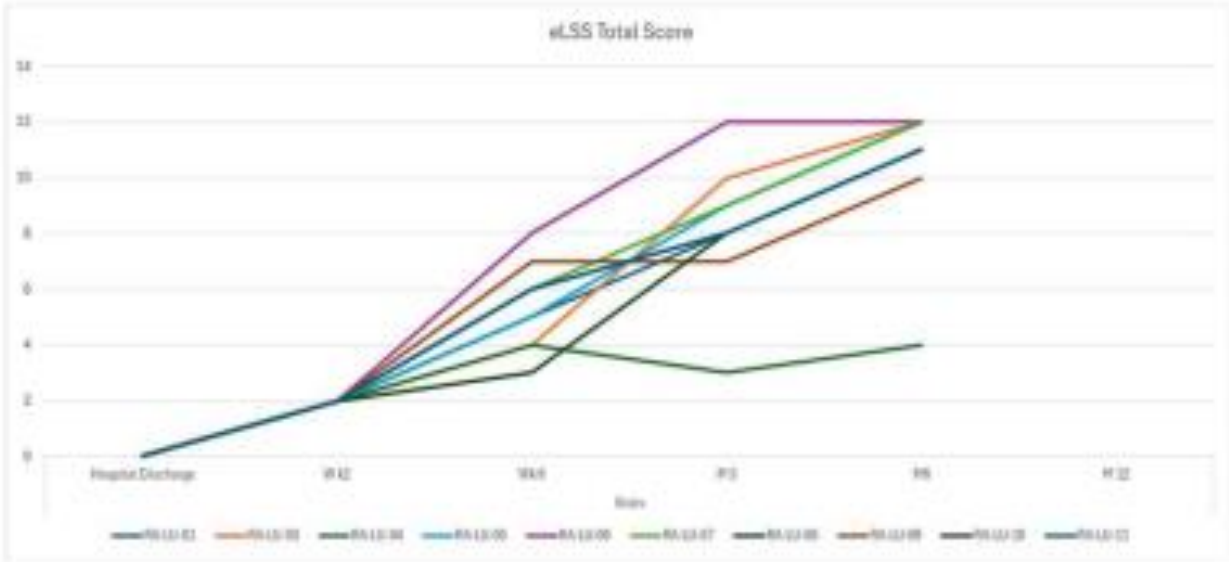
Modified MAYO Wrist Score

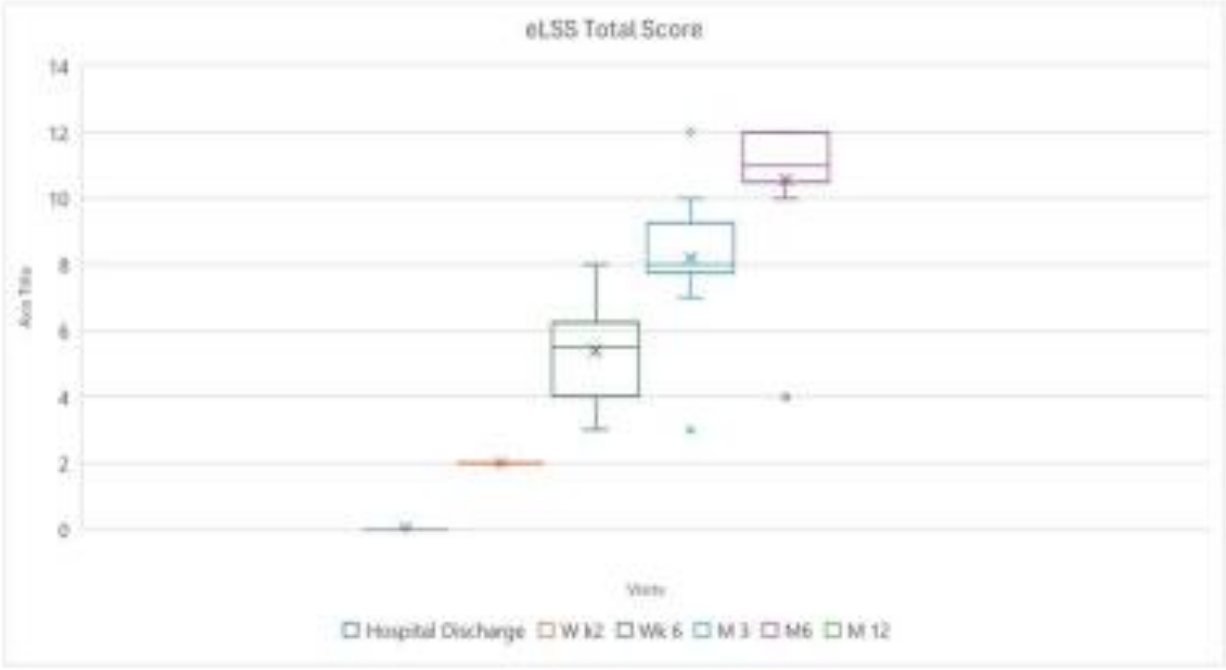
Assess pain, the active flexion/extension arc (compared to the contralateral side), grip strength (compared to the contralateral side), and the ability to return to regular employment or activities.



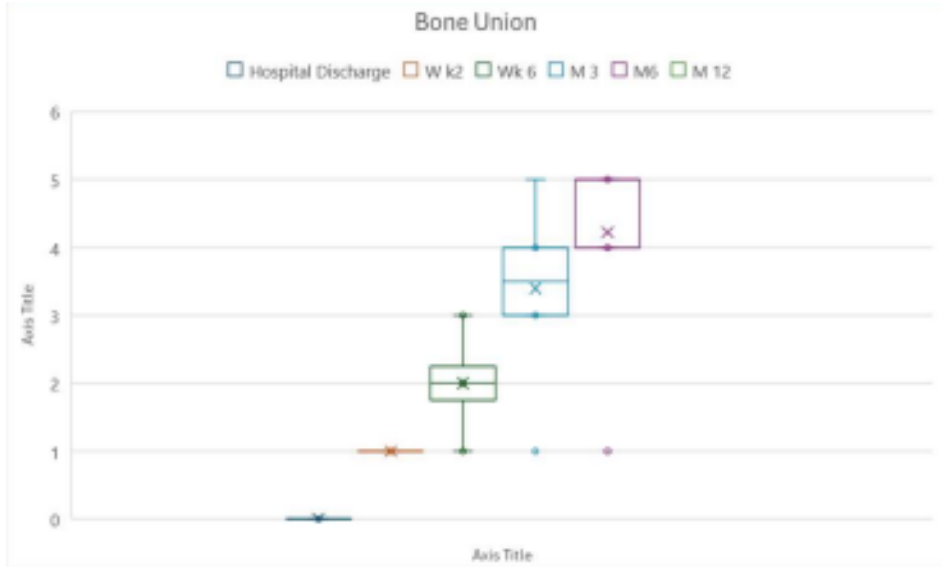
< 65 Poor
65-79 = Fair
80-89 = Good
90-100 = Excellent

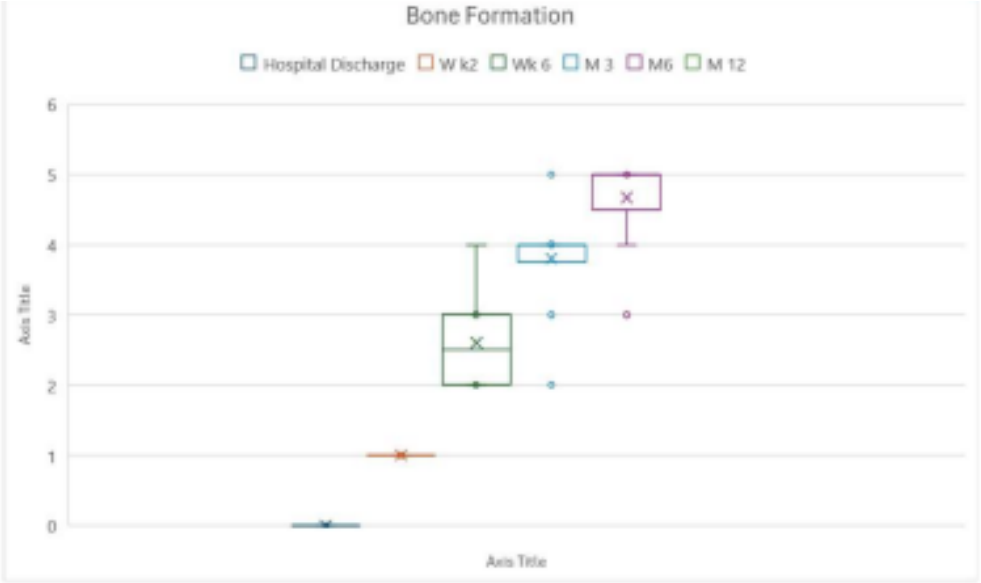
eLSS (x-ray 2 weeks, 6 weeks and 6 months





eLSS components





NVDX3 – Radius Safety

So far, a total of 58 Adverse Events were observed in 10 patients

- Treatment Emergent Adverse Events (TEAEs) = 43
  - *(Preliminary: AEs started prior to surgery not counted)*
- 1 SAE + AESI has been reported
  - *Brest cancer (tumor formation)*
- Only 1 AE (pain due to osteosynthesis – right wrist) was reported as being "definitely related" to NVDX3
  - *Also to the device*
- The severity was reported as mild (52/58), moderate (3/58) or severe (3/58)

Summary

NVD-X3

- an of-the-shelf-product
- Osteoinductive and osteoconductive
- Succesfully tested in distal radius fractures