Anti-RANKL Nanobody® ALX-0141 Shows Sustained Biomarker Inhibition in a Phase I Study in Healthy Postmenopausal Women

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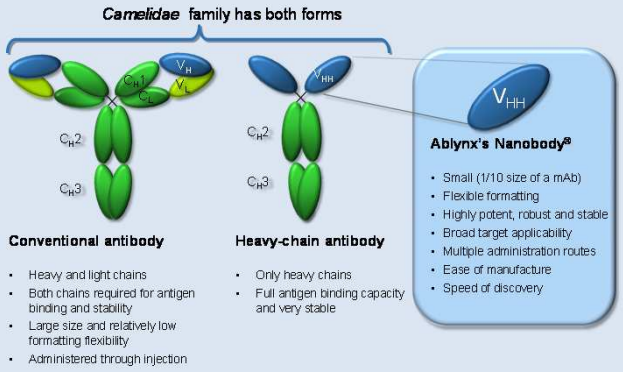
Summary

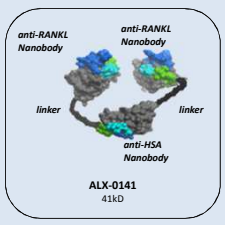
- Introduction: The interaction between RANK/RANKL is critical for the regulation of osteoclastogenesis and bone resorption. Inhibition of this interaction helps restore the balance between bone resorption and formation. ALX-0141, a novel biological agent (Nanobody) that specifically targets RANKL, was studied in a Phase I trial to assess the safety, tolerability, immunogenicity and PK after single injection.
- Methods: Forty-two healthy postmenopausal women (53-77 years, mean 66 years) were included in this study, which was approved by the local Ethical Committee. Participants received a single SC injection of ALX-0141 (n=31) at 6 dose levels, ranging from 0.003 to 1 mg/kg, or placebo (n=11). PK, PD and safety parameters were monitored for 3 months at the lowest dose level and for more than a year in the higher dose levels.
- Results: The safety analysis indicated that ALX-0141 was well tolerated. No serious adverse events related to ALX-0141 or dose-limiting toxicity occurred. The frequency of treatment emergent adverse events (TEAE) was similar in placebo-treated subjects (16 events in 7 subjects [64%]) and in subjects treated with ALX-0141 (93 events in 23 subjects [74%]). The most frequent TEAE were musculoskeletal and connective tissue disorders (n=27, reported by 14 subjects) and all TEAE were transient, of mild intensity, and did not result in any study withdrawals. ALX-0141 showed a favourable PK profile, triggering a prolonged PD response. Serum levels of the lead biomarker for bone resorption cross-linking telopeptide of type 1 collagen (CTx-1) decreased rapidly and remained suppressed for up to 390 days after a single SC administration of 1 mg/kg.
- Conclusions: The results from this Phase I trial indicate that ALX-0141 is a potent RANKL inhibitor that is well tolerated over a wide range of doses. This data supports the further development in bone-resorptive diseases with reduced BMD and increased fracture risk, such as in cancer-related bone diseases, osteoporosis and other disorders.

Ablynx's Nanobodies and ALX-0141



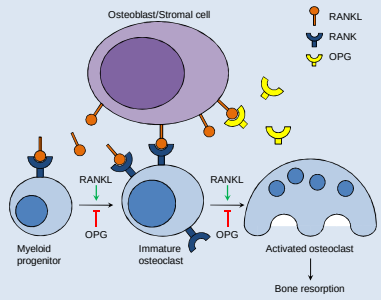
ALX-0141

- Two Nanobody units targeting RANKL, combined with a Nanobody targeting HSA
  - relative small size of ALX-0141
- Highly potent through bivalent target binding
- Serum albumin targeting
  - provides half-life extension
  - distribution throughout the body
  - penetration of inflamed/cancerous regions
- Highly stable, can be formulated at high concentrations for administration via subcutaneous (SC) injection
- Manufactured at high titres in a microbial production system



RANKL and its inhibition

- RANKL is mediator of osteoclastic bone resorption
- RANKL binding to RANK mediates differentiation, increased activity and survival of osteoclasts
- OPG is a natural antagonist
- Anti-RANKL Nanobody inhibits pathway via interference of RANKL binding to RANK



Preclinical data with ALX-0141

Variable	Test system	Result
Mode of action	Inhibition ELISA	Inhibition of binding
	Cell-based assay on RANKL-driven osteoclastogenesis	Inhibition of RANKL/ RANK interaction
Affinity	RANKL (SPR)	KD = 0.04 nM
	HSA (SPR)	KD = 10.4 nM
Potency (*in vitro*)	Inhibition ELISA	IC50 = 53 pM
	Soluble (s) RANKL-induced osteoclastogenesis	IC50 = 62 pM
	sRANKL-induced osteoclasto-genesis of $CD14^+$ monocytes	IC50 = 85 pM
	RANKL-induced osteoclastogenesis	IC50 = 34 pM
Specificity	ELISA with 7 TNF-family proteins	No cross-reaction
	Immuno-histochemistry on tissues	Confirms RANKL expression pattern
Potency (*in vivo*)	Effect on biochemical markers: CTx-1, TRACP5b, P1NP, Ca/P/PTH	CTx-1 inhibition: IC50= 0.54 nM

Study design and demographics

Phase I study design and treatment schedule

- Healthy postmenopausal woman [n=42]
  - 0.003 mg/kg ALX-0141 [n=1], randomised 1:1 to placebo
  - 0.01 mg/kg ALX-0141 [n=6]
  - 0.03 mg/kg ALX-0141 [n=6]
  - 0.1 mg/kg ALX-0141 [n=6]
  - 0.3 mg/kg ALX-0141 [n=6]
  - 1 mg/kg ALX-0141 [n=6]

  } randomised 3:1 to placebo

Objectives

- Determine MTD/BED
- Determine safety and tolerability of single SC doses

Key inclusion criteria

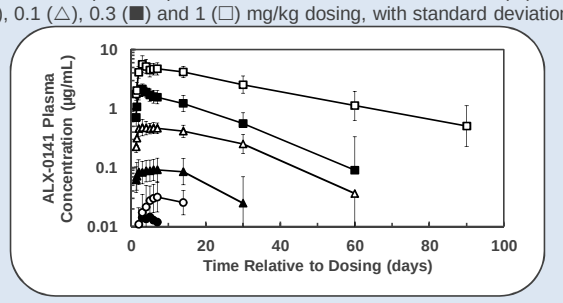
- Healthy postmenopausal women ≤ 80 years
- BMI of 18-36 kg/m²
- Normal lab parameters
- No history of relevant allergies
- No use of concomitant medication

Treatment	N	Age (years)		Weight (kg)		BMI (kg/m²)	
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Placebo	11	67 (3)	61-70	69.6 (6.5)	56.6-77.3	26.0 (1.9)	22.4-28.4
0.003 mg/kg	1	53 (-)	53-53	60.2 (-)	60.2-60.2	22.7 (-)	22.7-22.7
0.01 mg/kg	6	66 (5)	56-71	63.9 (6.7)	53.8-71.9	24.0 (2.5)	21.6-28.1
0.03 mg/kg	6	68 (7)	60-77	69.8 (6.0)	61.7-79.4	26.1 (2.0)	23.5-29.2
0.1 mg/kg	6	66 (6)	59-74	74.4 (10.3)	63.3-88.9	26.3 (2.3)	23.0-29.4
0.3 mg/kg	6	61 (6)	55-71	69.6 (12.2)	55.9-82.4	26.9 (3.1)	23.3-30.8
1 mg/kg	6	68 (3)	64-72	68.8 (9.4)	56.9-83.1	25.5 (2.6)	23.1-30.2
Overall	42	66 (5)	53-77	69.2 (8.5)	53.8-88.9	25.7 (2.4)	21.6-30.8

Pharmacokinetics (PK)

Geometric mean plasma concentrations of ALX-0141

ALX-0141 was administered on Day 1 and plasma concentrations were determined at multiple time points. Geometric mean data for 0.003 (●), 0.01 (○), 0.03 (▲), 0.1 (△), 0.3 (■) and 1 (□) mg/kg dosing, with standard deviation.



Summary of PK parameters

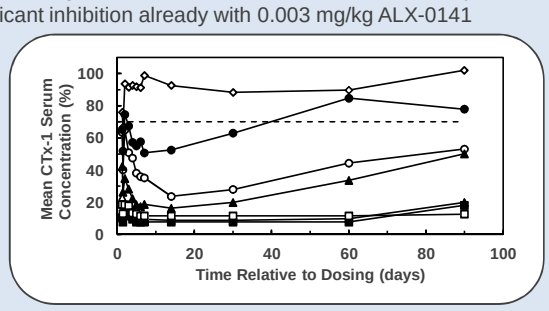
Treatment	N	C_{max} (µg/ml)	t_{max} (d)	AUC_{last} (µg.d/ml)	AUC_{inf} (µg.d/ml)	$t_{½}$ (d)
0.003 mg/kg	1	0.015 (-)	4.0 (-)	0.061 (-)	*	*
0.01 mg/kg	6	0.033 (0.016-0.067)	6.0 (5.0-13.2)	0.32 (0.14-0.66)	*	*
0.03 mg/kg	6	0.11 (0.06-0.18)	3.0 (0.3-13.0)	1.8 (0.7-3.4)	1.5 #	8.9 #
0.1 mg/kg	6	0.54 (0.42-0.71)	2.0 (1.0-13.0)	14.7 (8.2-21.9)	16.0 (11.6-22.3)	12.0 (9.8-14.8)
0.3 mg/kg	6	2.24 (1.72-2.93)	1.5 (1.0-2.0)	43.3 (25.3-67.1)	44.3 (25.8-68.0	12.4 (8.1-18.5)
1 mg/kg	6	5.76 (3.84-9.03)	2.0 (2.0-3.0)	193 (113-303)	200 (115-304)	20.6 (13.8-31.6)

For each subject, PK data was described according to a non-compartmental analysis revealing the C_{max}, t_{max}, AUC_{last}, AUC_{inf} and $t_{½}$. Geometric means and ranges are presented, except for t_{max}, which represents the median with range.
* No descriptive statistics possible; # Single observation

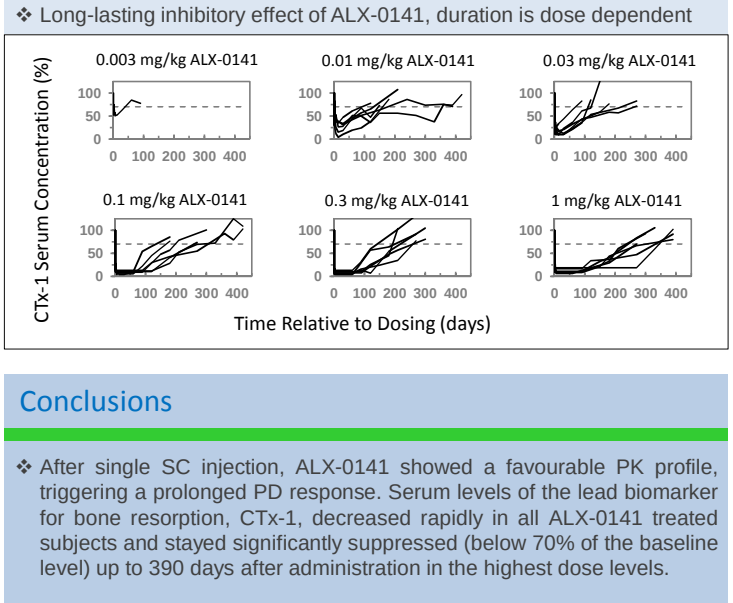
Pharmacodynamics (PD) – CTx-1

Mean CTx-1 serum concentrations: Marker for osteoclast activity

- Rapid and significant decrease of CTx-1 within 8 hours post-dose
- Significant inhibition already with 0.003 mg/kg ALX-0141



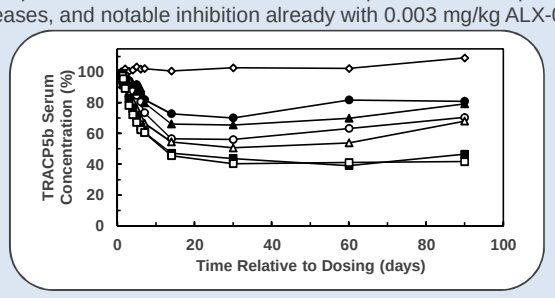
- Long-lasting inhibitory effect of ALX-0141, duration is dose dependent



Other PD markers

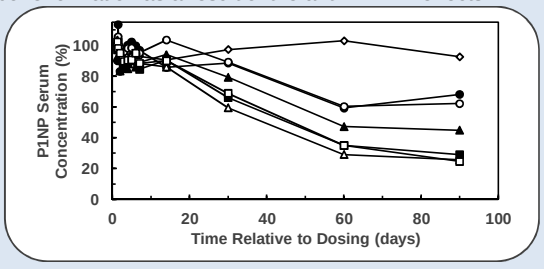
Mean tartrate-resistant acid phosphatase type 5b (TRACP5b) concentrations: Marker for osteoclast numbers

- Similar profiles as for serum CTx-1 levels: rapid and dose-dependent decreases, and notable inhibition already with 0.003 mg/kg ALX-0141



Procollagen type 1 N-terminal propeptide (P1NP): Marker for osteoblast activity

- Less bone formation as a result of the anti-RANKL effects



Safety and tolerability

Summary of TEAE by relationship and intensity

- 109 TEAE described by 30 subjects, mostly mild in intensity
- Majority were not considered related to study medication
- The most frequent TEAE considered to be related were muscle spasms and musculoskeletal stiffness, which were potentially related to low calcium levels, expected as a results of the pharmacological effect of ALX-0141
- Most TEAE were transient and resolved at the time of last visit; 11 were on-going. These were considered remotely or not-related.

Treat-ment	N	Mild				Moderate				Severe				Total					
		Related		Not Related		Related		Not Related		Related		Not Related		Related		Not Related		All	
		e	n	e	n	e	n	e	n	e	n	e	n	e	n	e	n	e	n
Placebo	11	1	1	15	6	0	0	0	0	0	0	0	0	1	1	15	6	16	7
0.003 mg/kg	1	0	0	7	1	0	0	0	0	0	0	0	0	0	0	7	1	7	1
0.01 mg/kg	6	0	0	4	4	0	0	0	0	0	0	0	0	0	0	4	4	4	4
0.03 mg/kg	6	5	3	18	5	0	0	1	1	0	0	1	1	5	3	20	5	25	5
0.1 mg/kg	6	4	2	11	4	0	0	0	0	0	0	0	0	4	2	11	4	15	5
0.3 mg/kg	6	1	1	6	2	0	0	0	0	0	0	0	0	1	1	6	2	7	3
1 mg/kg	6	2	2	33	5	0	0	0	0	0	0	0	0	2	2	33	5	35	5
Total	42	13	9	94	27	0	0	1	1	0	0	1	1	13	9	96	27	109	30

Abbreviations: 'N' denotes the numbers of subjects per cohort, 'e' is the number of times a TEAE occurred per cohort, and 'n' is the number of subjects that experienced at least one TEAE per cohort

Severe advents

- Two SAE occurred in 2 subjects, a Lipitor® induced pancreatitis and dental implant inflammation; both were considered not related to ALX-0141 treatment

Conclusions

- After single SC injection, ALX-0141 showed a favourable PK profile, triggering a prolonged PD response. Serum levels of the lead biomarker for bone resorption, CTx-1, decreased rapidly in all ALX-0141 treated subjects and stayed significantly suppressed (below 70% of the baseline level) up to 390 days after administration in the highest dose levels.
- The safety analysis indicated that ALX 0141 was well tolerated. No serious adverse events or dose-limiting toxicity occurred. There were no significant differences in the frequencies and severities of adverse events for subjects receiving ALX-0141 compared with placebo-treated subjects. All treatment related adverse events were transient, of mild intensity, and did not result in any study withdrawals.
- The results from this Phase I trial indicate that ALX 0141 is a potent RANKL inhibitor and can be administered over a wide range of doses. Collectively, this data supports the further development of ALX-0141 in bone-resorptive diseases involving reduced bone mineral density and increased fracture risk, such as in cancer-related bone diseases, osteoporosis and other disorders.

Footnotes

- Acknowledgement: This work is the result of the efforts of many individuals within Ablynx – The authors gratefully thank everyone who contributed in any form or way.
- Conflict of interest: All authors are employees of Ablynx. Ablynx was involved in designing the study plan, analysis of the data and generation of the study report.