

**Low Molecular Weight Heparins:
Varied Biologics with Differing
Structures, Activities, and
Clinical Effects**

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Differences in Clinical Development Plans & Outcomes are Reflected in Labels

Indication	Enoxaparin	Dalteparin	Nadroparin	Fondaparinux
DVT/PE Treatment	●	●	●	●
Treatment of NSTEMI	●	●		To be filed
DVT Prevention in Orthopaedic*	●	●	●	●
Long Term DVT Prevention in Orthopaedics *	●	●	●	●
DVT Prevention in Surgery	●	●	●	●
Hemodialysis	●	●	●	
DVT Prevention in Medical	●			
Treatment of STEMI	●			To be filed

Regulatory Agencies & Clinical Guidelines Regard LMWH's as Distinct and Non-Interchangeable Drugs

▶ Agencies & Health Organizations

- FDA: « ...most particularly to the fact that LMWHs cannot be used interchangeably, unit for unit, with heparin, nor can one individual **LMWH be used interchangeably with another.**»
- WHO: "Because LMWHs are prepared by different methods of depolymerisation, they differ to some extent in pharmacokinetic properties and anticoagulant profiles, and **are not clinically interchangeable**" WHO Headquarters, Geneva, Switzerland, 7-8 September 1999

▶ Guidelines

- ACCP: "Because LMWHs are prepared by different methods of depolymerisation, they differ to some extent in pharmacokinetic properties and anticoagulant profiles, and **are not clinically interchangeable**" Geerts WH, et al. *Chest*. 2004;126(Suppl 3): 338S-400S.
- AHA / ACC: "Although LMWHs share many pharmacological similarities, they also vary in important respects, and it is important **to consider each drug individually rather than as members of interchangeable compounds.**" Ryan TJ, et al. *J Am Coll Cardiol* 1999;34:890-911
- ICS: « ...Therapeutic **interchange among these products is not appropriate.** The choice of LMWH should reflect the level of clinical evidence and the approval of the regulatory authorities for each indication." Nicolaidis AN, et al. *Int Angiol*. 2006;25:101-61

What are the Characteristics of a Well Defined LMWH?

- ▶ **Reproducibility** of pharmacological activities beyond anti Xa- and anti IIa- activities despite
 - High complexity of molecular structure
 - Biological origin
- ▶ **Reliability** of its clinical use in any different clinical setting
 - Studied in sufficiently large patient populations
 - Studied in special patient populations

Structural modifications of LMWH are highly sensitive to the manufacturing process

Structural Features Contributing to the Pharmacological Actions of LMWHs

- ▶ **Molecular weight distribution**
- ▶ **Oligosaccharide components**
- ▶ **Consensus sequences**
- ▶ **Degree of sulphation/charge density**
- ▶ **Presence of unique structural features (double bond, andromanno sugars)**

What Criteria are Relevant in Choosing an Innovator LMWH?

Chemical properties make each LMWH unique



This translates into **pharmacological** and potentially **immunogenic** differences among LMWHs

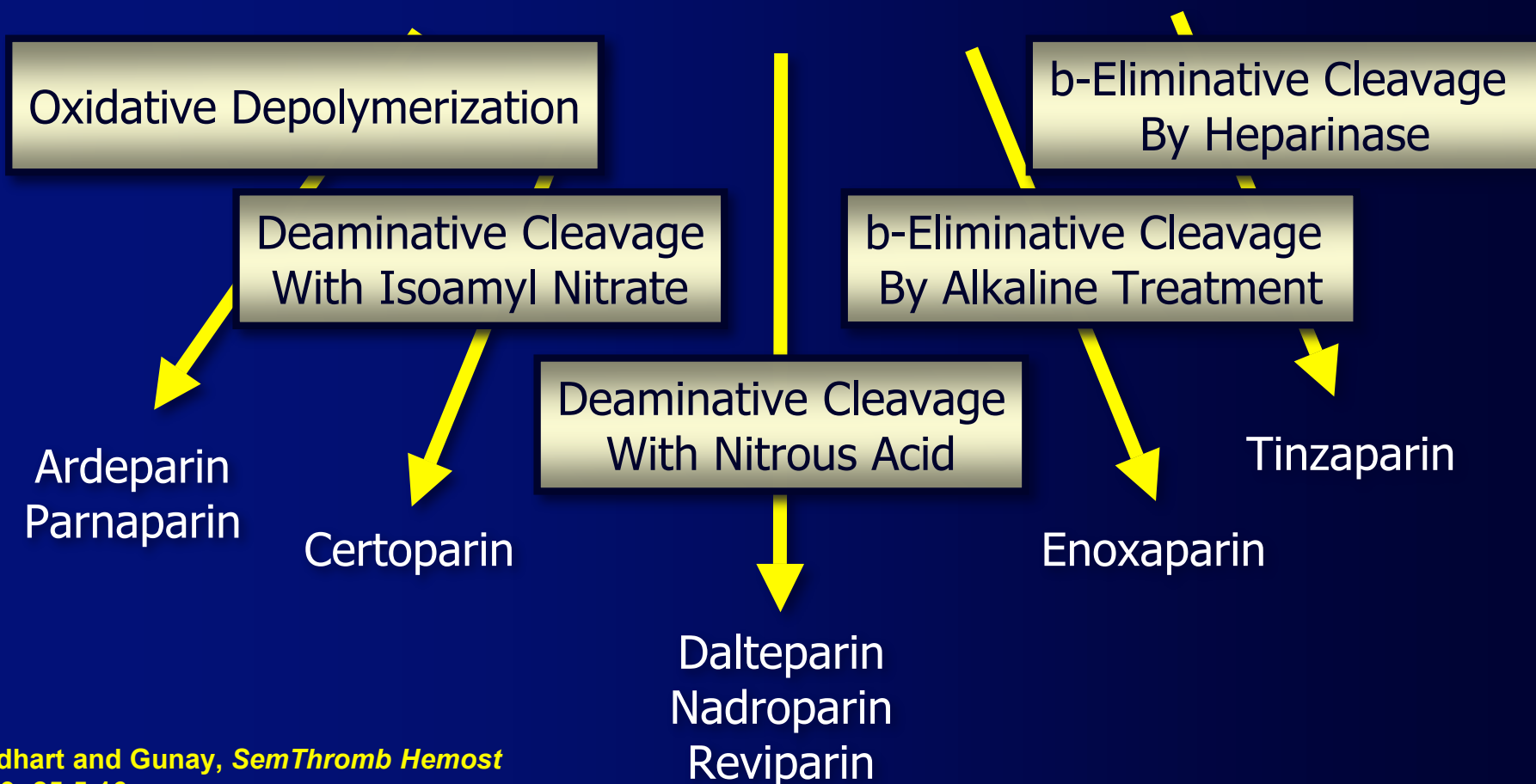


This impacts the **clinical** safety and efficacy in both arterial and venous thrombosis

The manufacturing process makes the product unique

LMWHs – Method of Preparation

Unfractionated Heparin

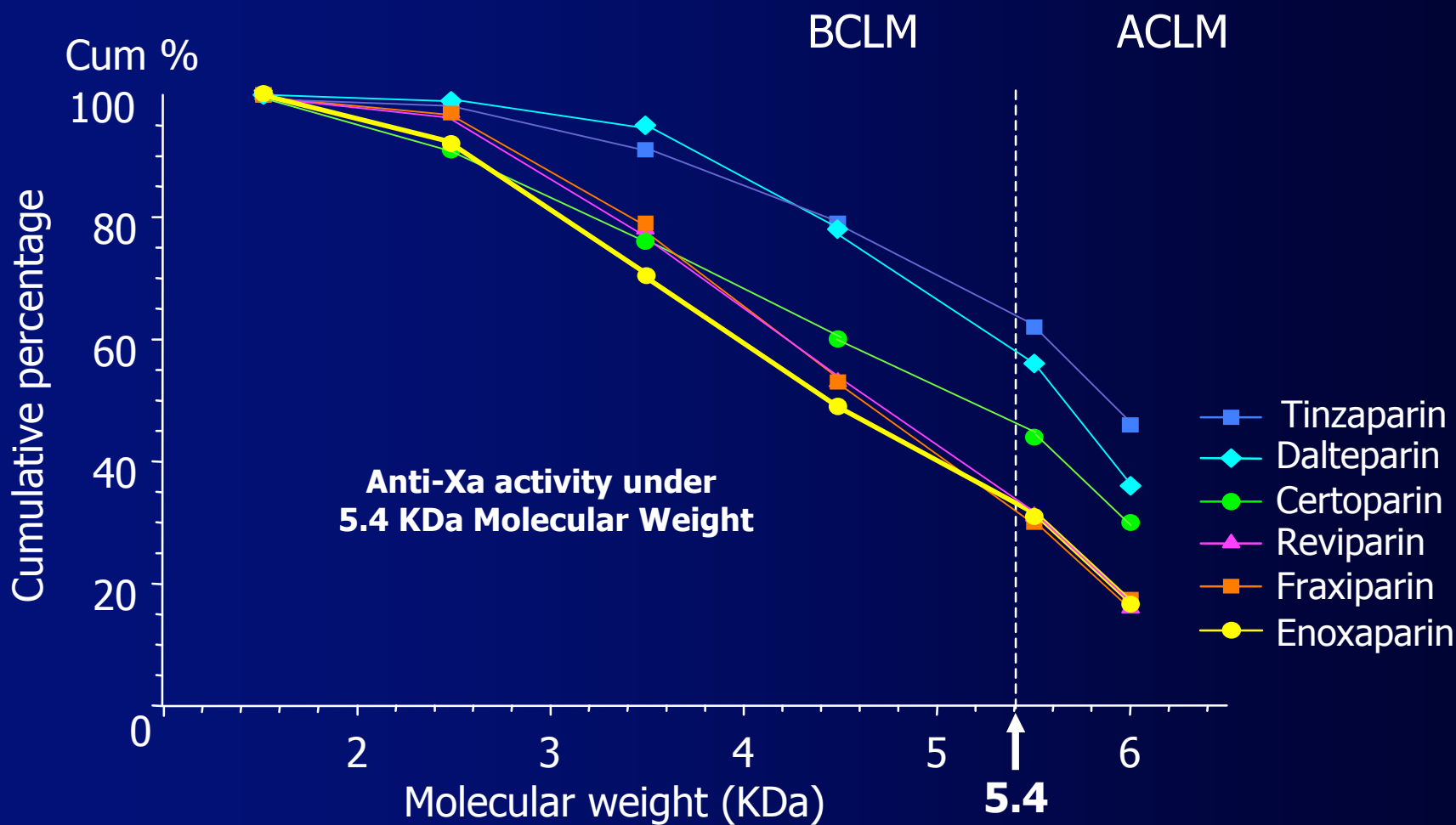


Molecular Weight Distribution

BCLM = below critical length material

ACLM = above critical length material

Da = Daltons



Characteristic Comparison

	SQ UFH	Enoxaparin	Dalteparin	Tinzaparin
F	20%	100%	87%	87%
T_{1/2}	~1 h	4.5 h	3-5 h	3.9 h
MW	~15 k	4.5 k	6.5 k	6.5 k
Anti Xa: Anti IIa	1.2:1 [†]	3.8:1 [†] or 14.0:1 [‡]	2.7:1 [†]	2.8:1 [†]
ΔvWF	++++	+	++++	????
↑TFPI	+	++++	++	++++

[†] in vitro ratio determination

[‡] in vivo ratio determination based on an enoxaparin dose of 1.5mg/kg

Weitz JI. *N Engl J Med* 1997;337:688.

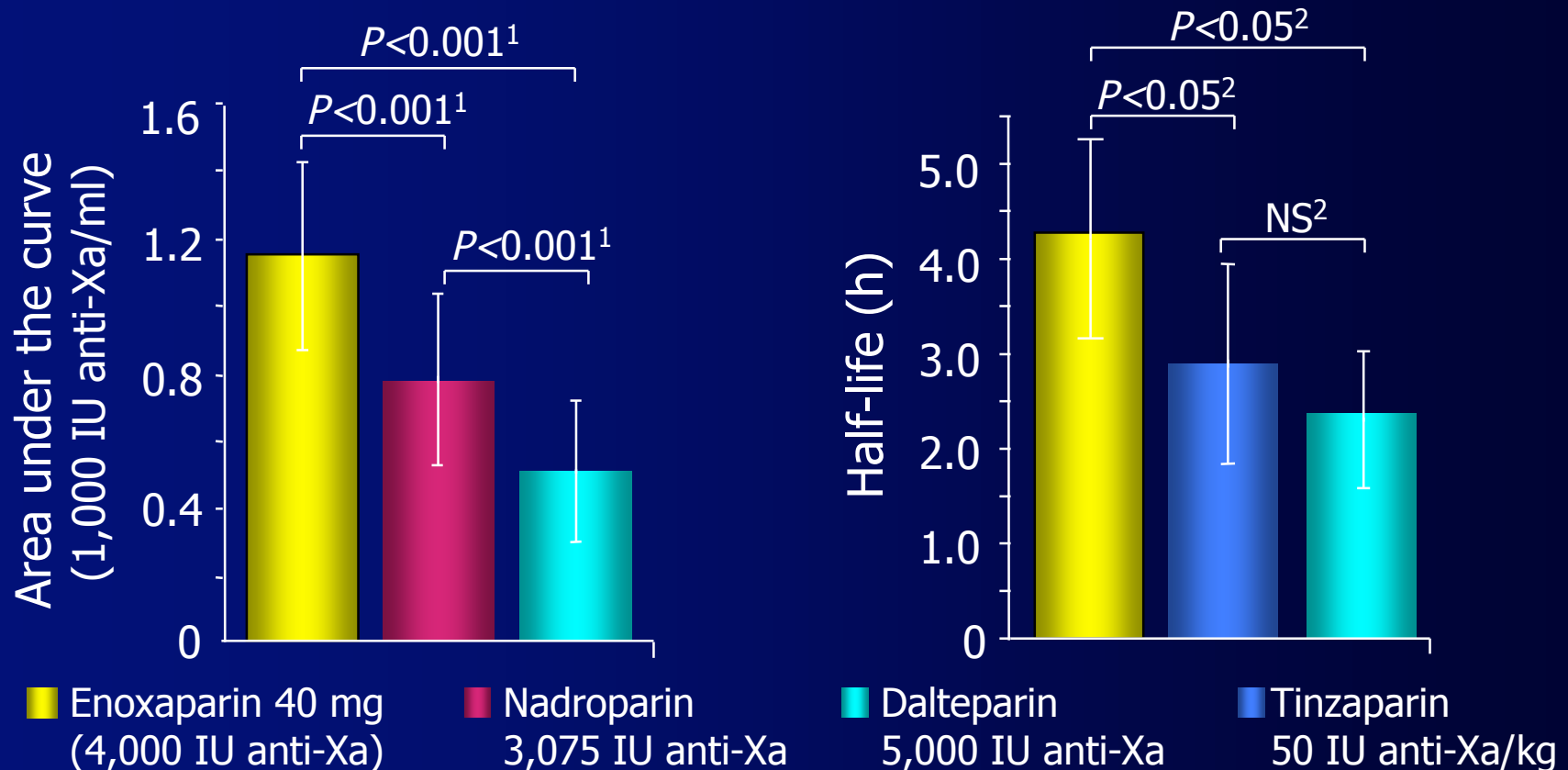
Montalescot G. *J Am Coll Cardiol* 2000;36:110-4.

Fareed J. *Ann NY Acad Sci* 1989;556:333-53

Lovenox Prescribing Information, Rev Sept 2006

Plasma Anti-Xa Following LMWH at Approved Doses for Prevention of Deep Vein Thrombosis

Currently employed LMWHs vary significantly

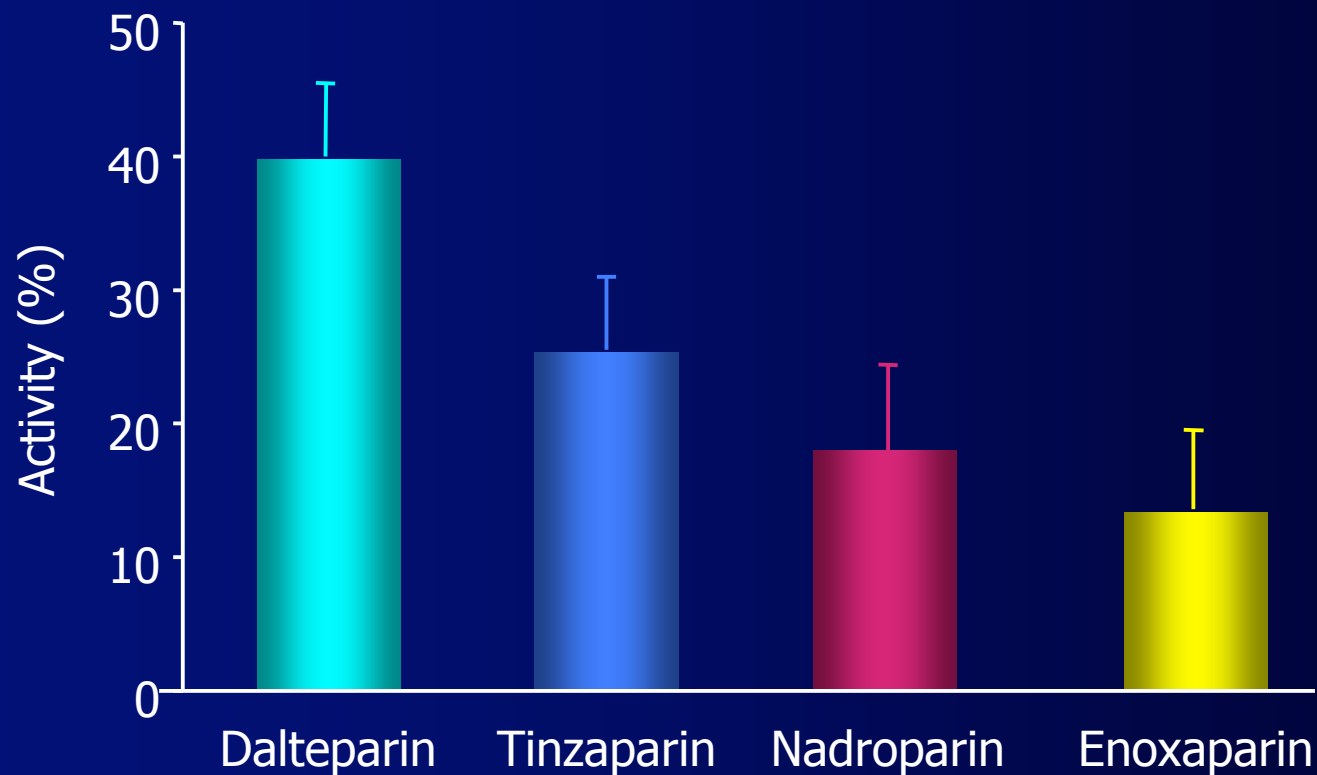


1. Collignon F, et al. *Thromb Haemost* 1995;73:2-12

2. Eriksson BI, et al. *Thromb Haemost* 1995;73:398-401

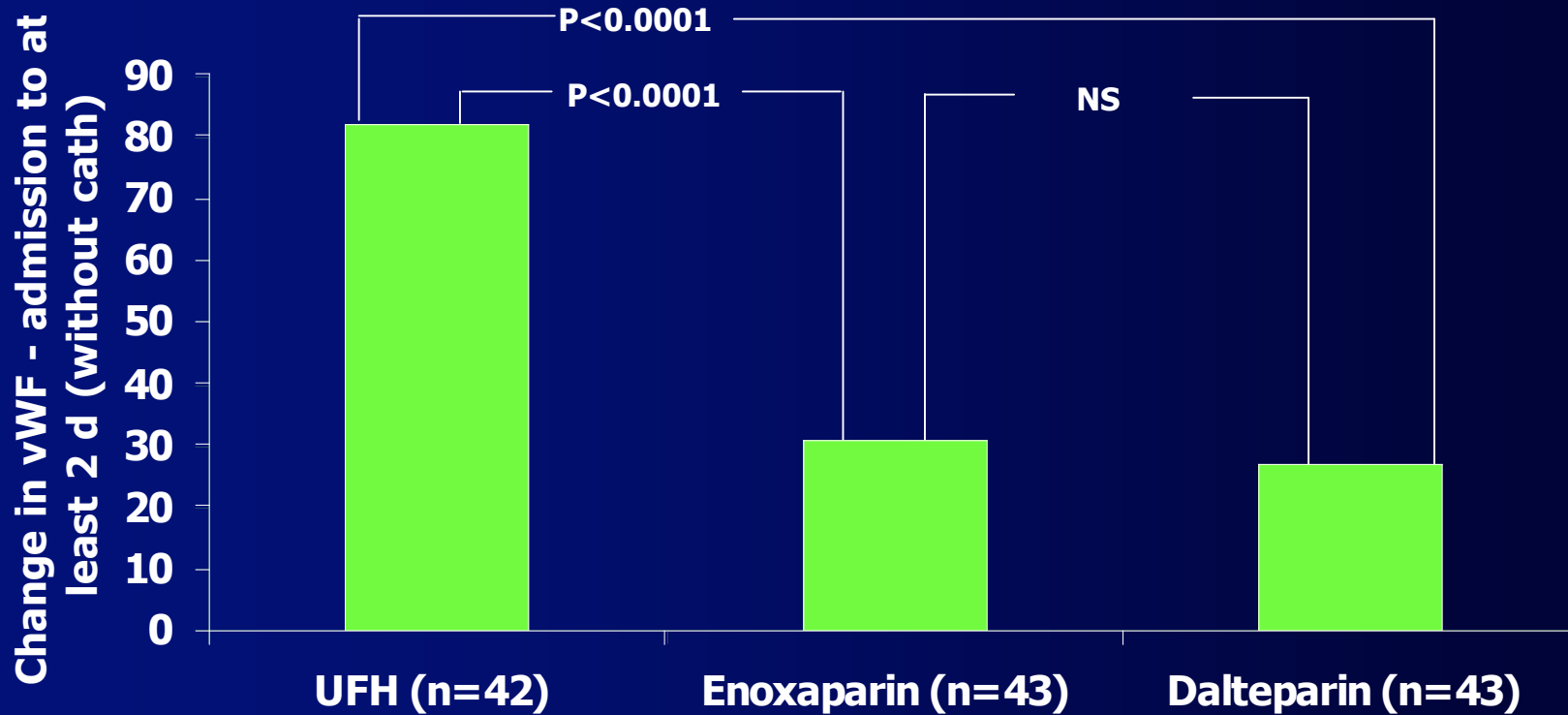
Effect of LMWHs on Thrombin Generation

Currently employed LMWHs vary significantly



ARMADA

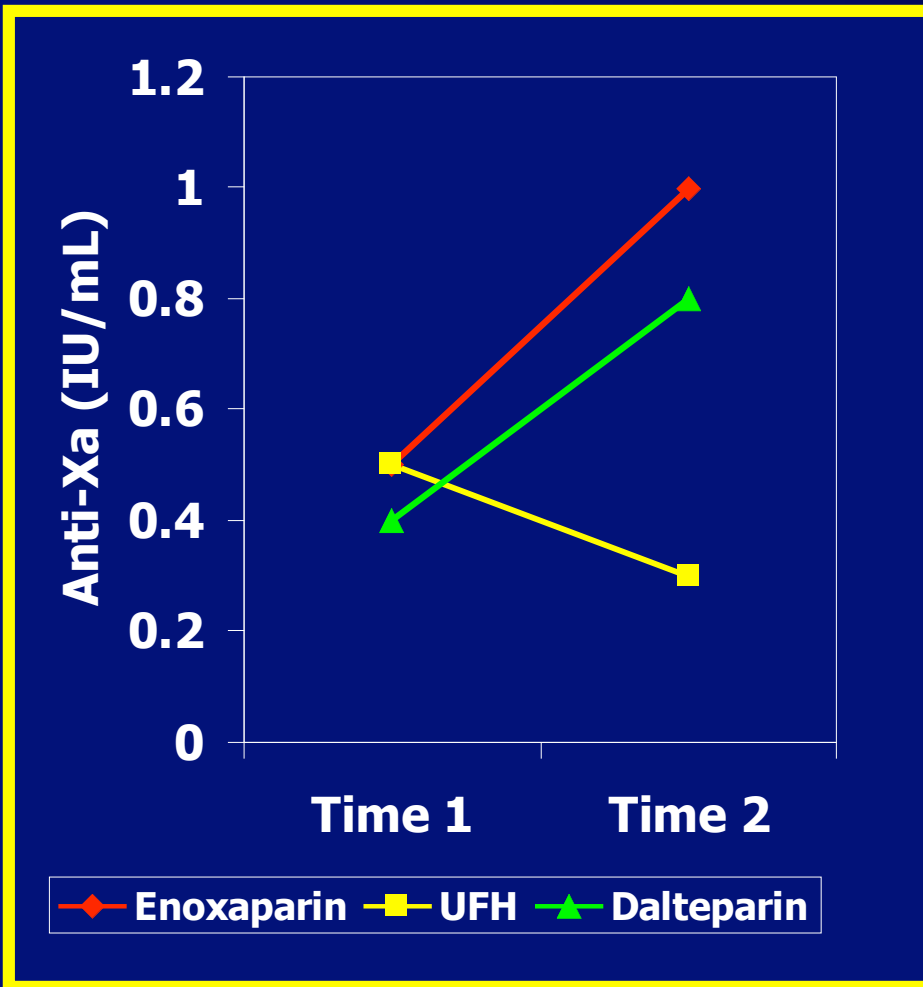
Δ vWF



Indep predictor - High Δ vWF (p=0.044)

ARMADA

Results



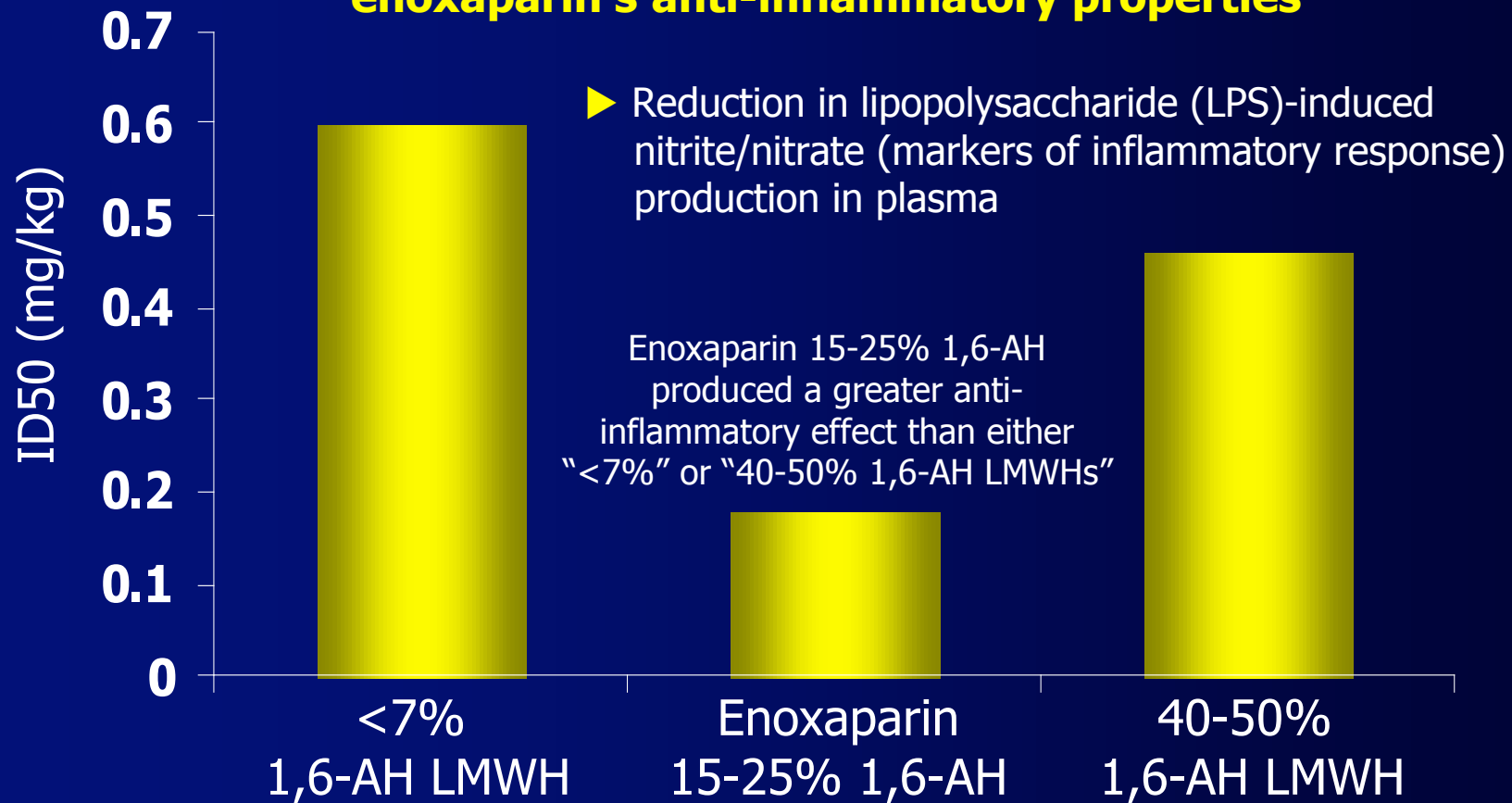
- ▶ Independent predictors of adverse outcome
 - NQMI on admission (p=0.045)
 - High Δ vWF (p=0.44)
 - Low Δ GPIIb/IIIa receptors in ADP stimulated PLT (p=0.005)

Innovator LMWH Differentiation: Clinically Relevant Attributes

- ▶ **Structural differences** **May impact PK/PD, immunogenicity**
- ▶ **Molecular size** **May impact anti-Xa, anti-IIa**
- ▶ **Charge density** **Interaction with cells**
- ▶ **Binding to ATIII** **Antithrombotic effects**
- ▶ **Binding to Heparin Cofactor II** **Anticoagulant effects**
- ▶ **Ability to release TFPI** **Inhibition of formed TF**
- ▶ **Interaction with proteins** **Decreased PK/PD**
- ▶ **Interactions with cells** **Signaling effects (not understood)**
- ▶ **Ability for glycosylation** **Biological amplification**
- ▶ **Vascular uptake** **Antithrombotic surface**
- ▶ **Endovascular uptake** **Inhibition of vascular proliferation**
- ▶ **Modulation of growth factors**..... **Anti-cancer, anti-apoptotic, anti angiogenic**
- ▶ **Molecular effects** **Genotypic expression changes**

Enoxaparin's unique 1,6-Anhydro Ring Effects on Inflammation

The presence of the 1,6- anhydro ring structure in enoxaparin at 15-25% concentration makes an important contribution to enoxaparin's anti-inflammatory properties



1,6-Anhydro Ring Effects: Reduced Smooth Muscle Cell Proliferation

▶ In-vitro experiments using enriched and depleted LMWH samples

Group 1 contained <7% of 1,6-AH LMWH

Group 2 contained 40–50% of 1,6-AH LMWH

- Group 1 vs enoxaparin: 35% less inhibitory action on smooth muscle cell proliferation**
- Group 2 vs enoxaparin: more potent inhibitor of smooth muscle cell proliferation**

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 - Studied in sufficiently large patient populations
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Structural modifications of LMWH are highly sensitive to the manufacturing process

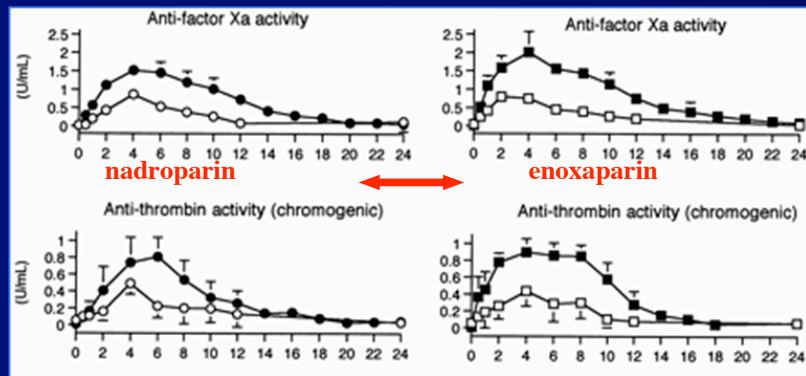
Marketed LMWH Do Not Differentiate Markedly for Common Parameters (anti-Xa, IIa, Ratio)

	Anti-Xa IU/mg	Anti-IIa IU/mg	Ratio Xa/IIa
Enoxaparin	104	32	3.3
Nadroparin	94	31	3.0
Tinzaparin	90	50	1.8
Dalteparin	122	60	2.0
Reviparin	130	40	3.3

- ▶ **Most of the differences remains within <10%:**
 - None of the in vitro biological activities considered alone enable differentiation
 - Even ratio would suggest enoxaparin=nadroparin=reviparin and tinzaparin=dalteparin
 - Nadroparin and enoxaparin would be similar on all parameters

80% to 125% Bioequivalence Margins are Unlikely to Differentiate Enoxaparin from other LMWHs or even UFH!

Would **enoxaparin** and **nadroparin** be bioequivalent at curative regimen on **anti-Xa** and **anti-IIa** Pharmacodynamic?



Salvioni A et al; Thromb Haemost 2001; 86: 991-4

Would **enoxaparin** and **UFH** be bioequivalent at curative regimen on **TAT, F1+2** Pharmacodynamic?

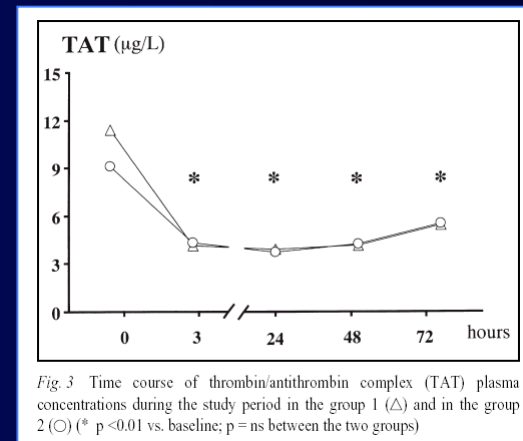
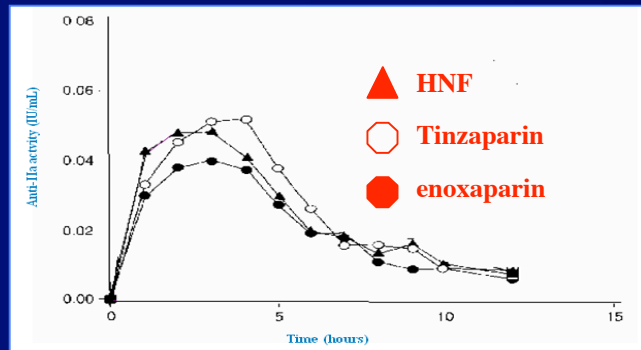


Fig. 3 Time course of thrombin/antithrombin complex (TAT) plasma concentrations during the study period in the group 1 (Δ) and in the group 2 (○) (* p < 0.01 vs. baseline; p = ns between the two groups)

Would **enoxaparin** and **tinzaparin** and **UFH** be bioequivalent at prophylactic regimen on **anti-IIa** Pharmacodynamic?



Adapted from Andrassy K et al; Thromb Research 1996 vol 81 n°2S:S29-S38 2001; 86: 991-4

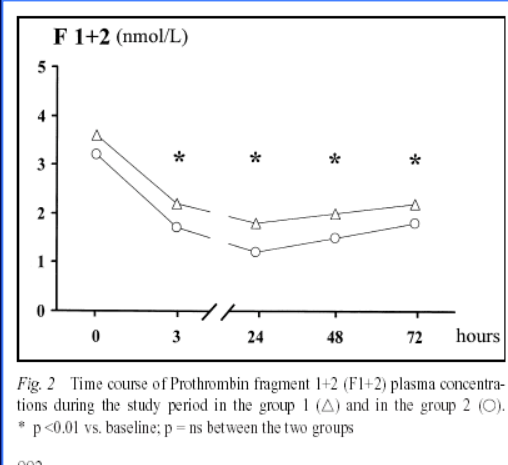
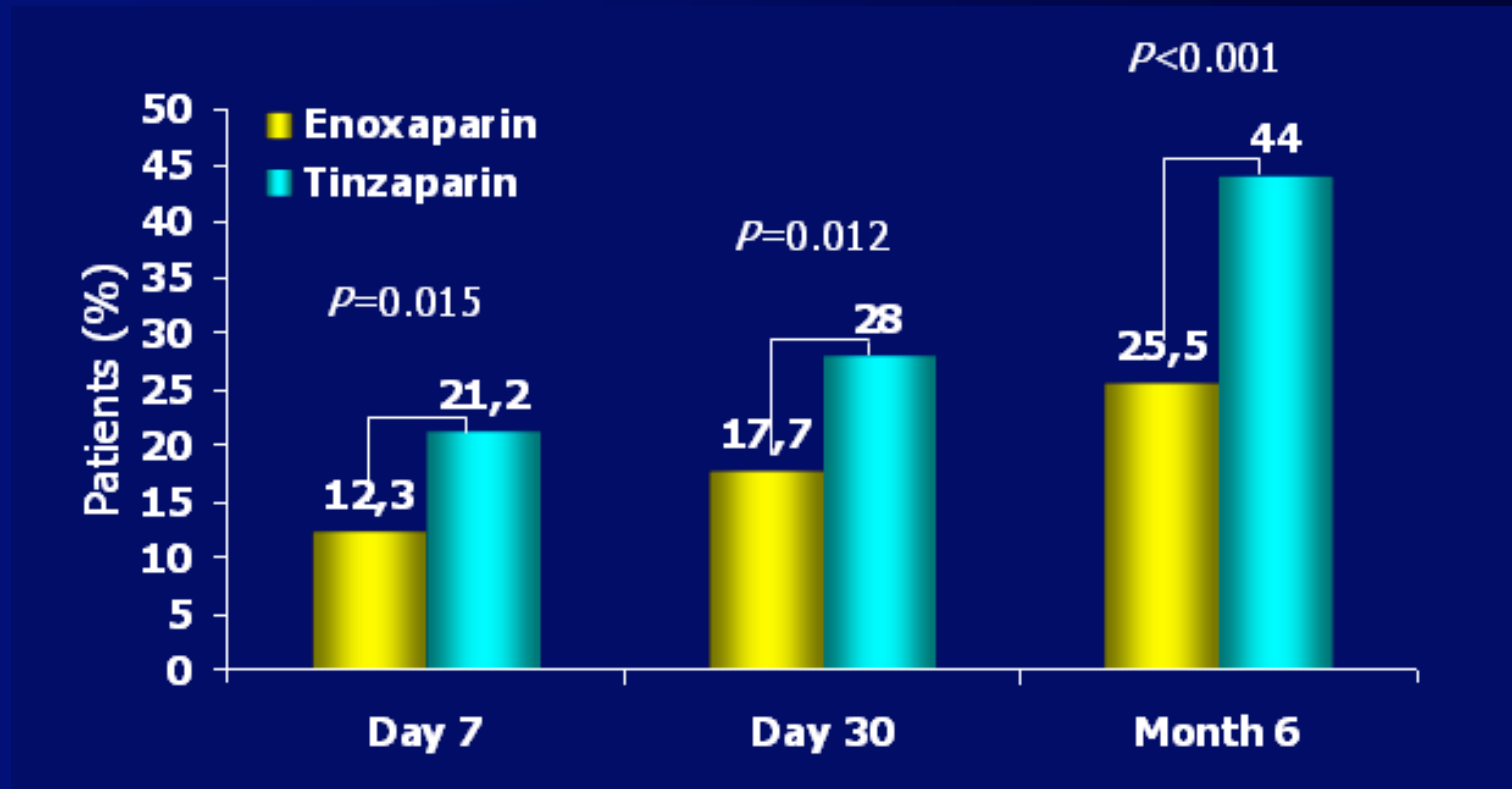


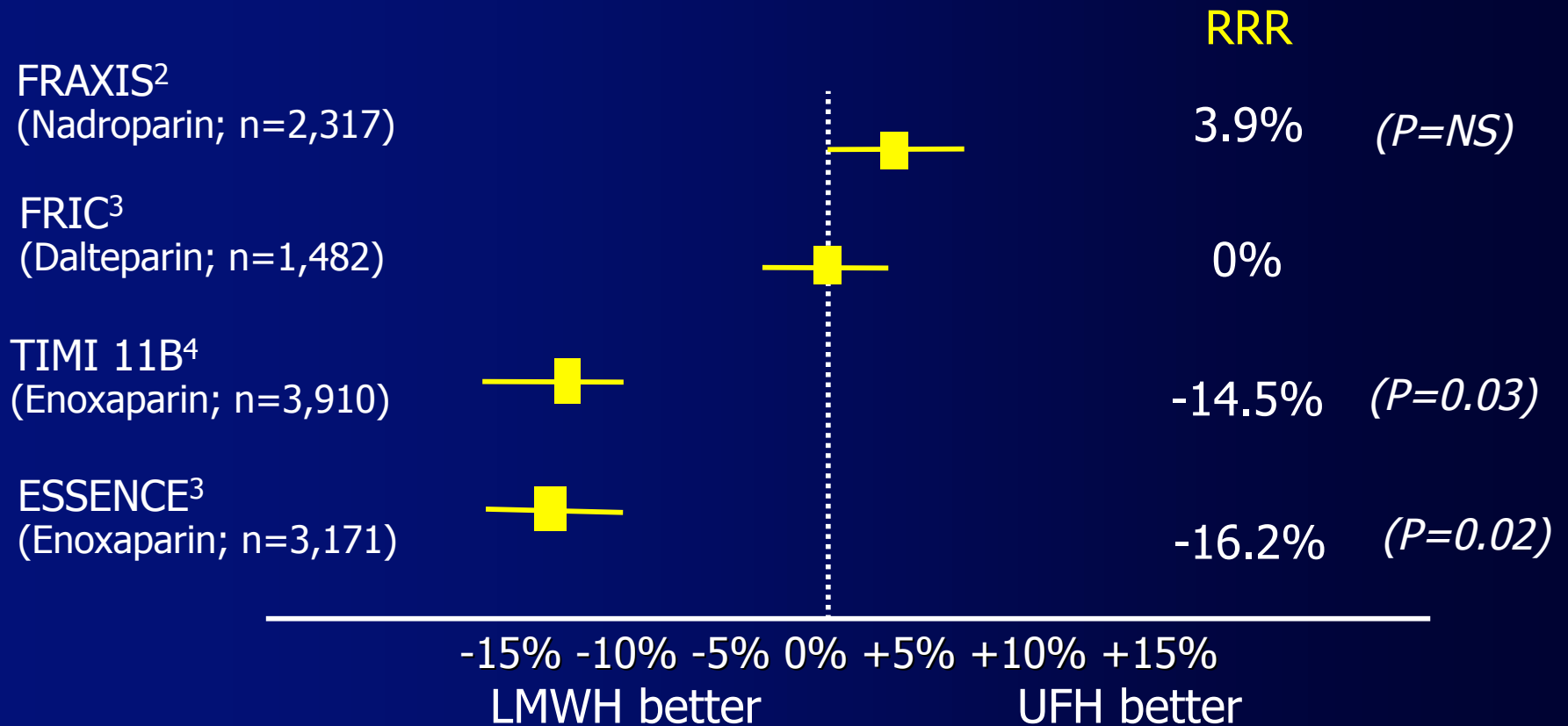
Fig. 2 Time course of Prothrombin fragment 1+2 (F1+2) plasma concentrations during the study period in the group 1 (Δ) and in the group 2 (○). * p < 0.01 vs. baseline; p = ns between the two groups

Differences Among Agents Compared Directly in NSTEMI ACS

EVET Composite Endpoint Death, MI, and Recurrent Angina



Differences Among Agents Compared Indirectly in NSTE ACS



*Triple endpoint: death, myocardial infarction, recurrent ischaemia ± urgent revascularization

Adapted from ¹Cohen M *et al.*, *J Am Coll Cardiol* 2003;41:55S– 61S

²*Eur Heart J* 1999;20:1553–62

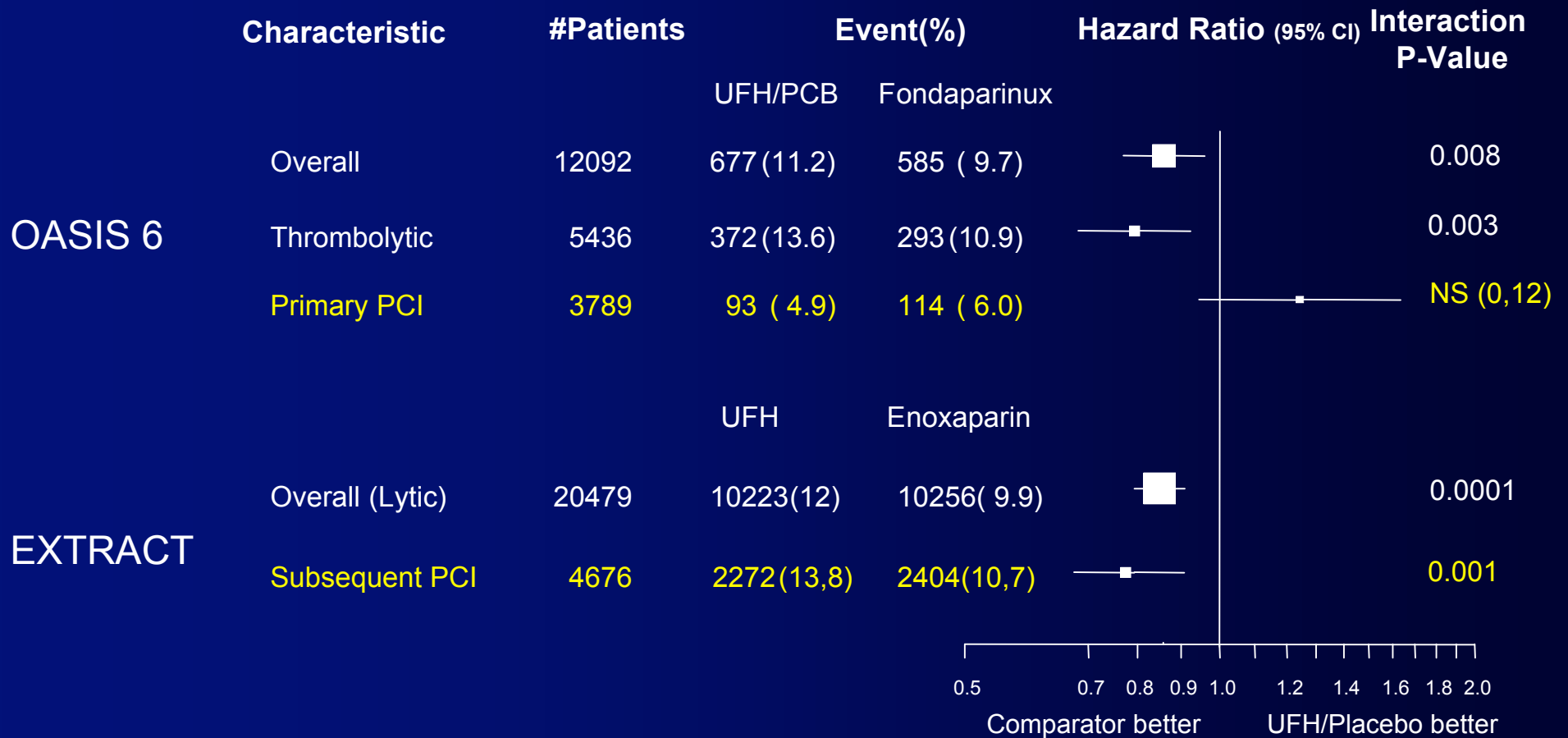
³Klein W *et al.*, *Circulation* 1997;96:61–8

³Antman EM *et al.*, *Circulation* 1999;100:1593–601

⁴Cohen M *et al.*, *N Engl J Med* 1997;337:447–52

Differences Between Heparins and Synthetic Anticoagulants

Primary Efficacy at 30 days

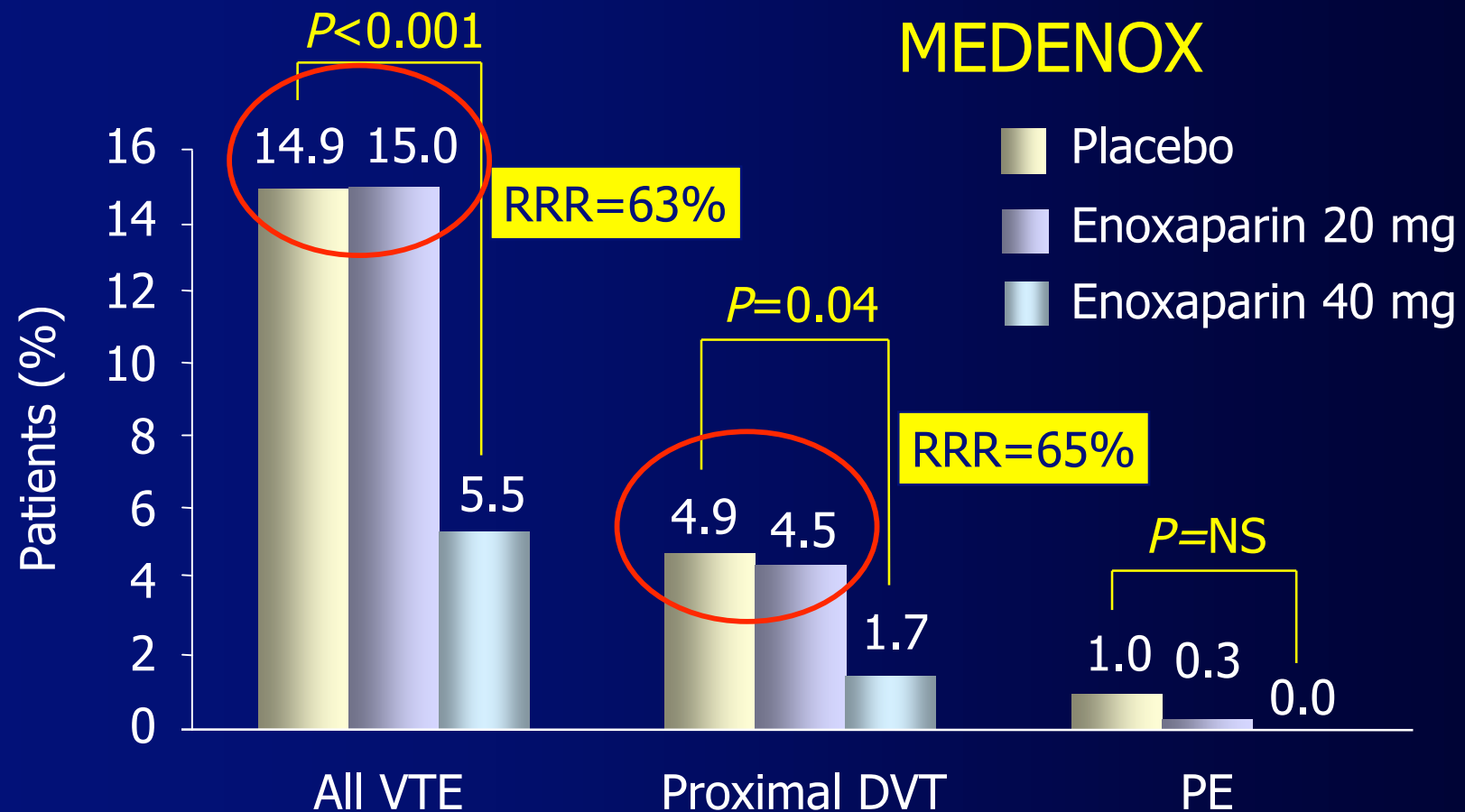


Differences Among Agents Compared Indirectly in VTE Prophylaxis

Study	Year	Thromboprophylaxis	Result in I ^{ary} endpoint†
MEDENOX	1999	Enoxaparin vs placebo	RRR = 63%; $p < 0,001$
PREVENT	2003	Dalteparin vs placebo	RRR = 45%; $p = 0.0015$
MAHE	2005	Nadroparin vs placebo	N=10.08%, PI=10.29%; $p = NS$

†The , MEDENOX, = All VTE assessed by venography - The PRIME = symptomatic VTE - Hillbom = Composite of VTE, Death, Intracranial bleeding, hemorrhagic transformation - PREVENT = All VTE assessed by US - MAHE = All cause mortality

Given these Concerns, How Would We Extrapolate Dosing to Another LMWH?



RRR = Relative risk reduction

LMWH vs Placebo in Medical Patients

Study	RRR	Thromboprophylaxis	Patients with VTE (%)
MEDENOX ¹ <i>P</i> <0.001	63%	Placebo	14.9* (n=288)
		Enoxaparin (40 mg q.d.)	5.5 (n=291)
PREVENT ² <i>P</i> =0.0015	45%	Placebo	5.0 (n=1,473) [†]
		Dalteparin (5,000 IU q.d.)	2.8 (n=1,518)
ARTEMIS ³ <i>P</i> =0.029	47%	Placebo	10.5 [‡] (n=323)
		Fondaparinux (2.5 mg q.d.)	5.6 (n=321)

*VTE at day 14; [†]VTE at day 21; [‡]VTE at day 15.

1. Samama MM, et al. *N Engl J Med*. 1999;341:793-800

2. Leizorovicz A, et al. *Circulation*. 2004;110:874-9

3. Cohen AT, et al. *Blood*. 2003;102:abstract 42

Enoxaparin vs UFH in Medical Patients

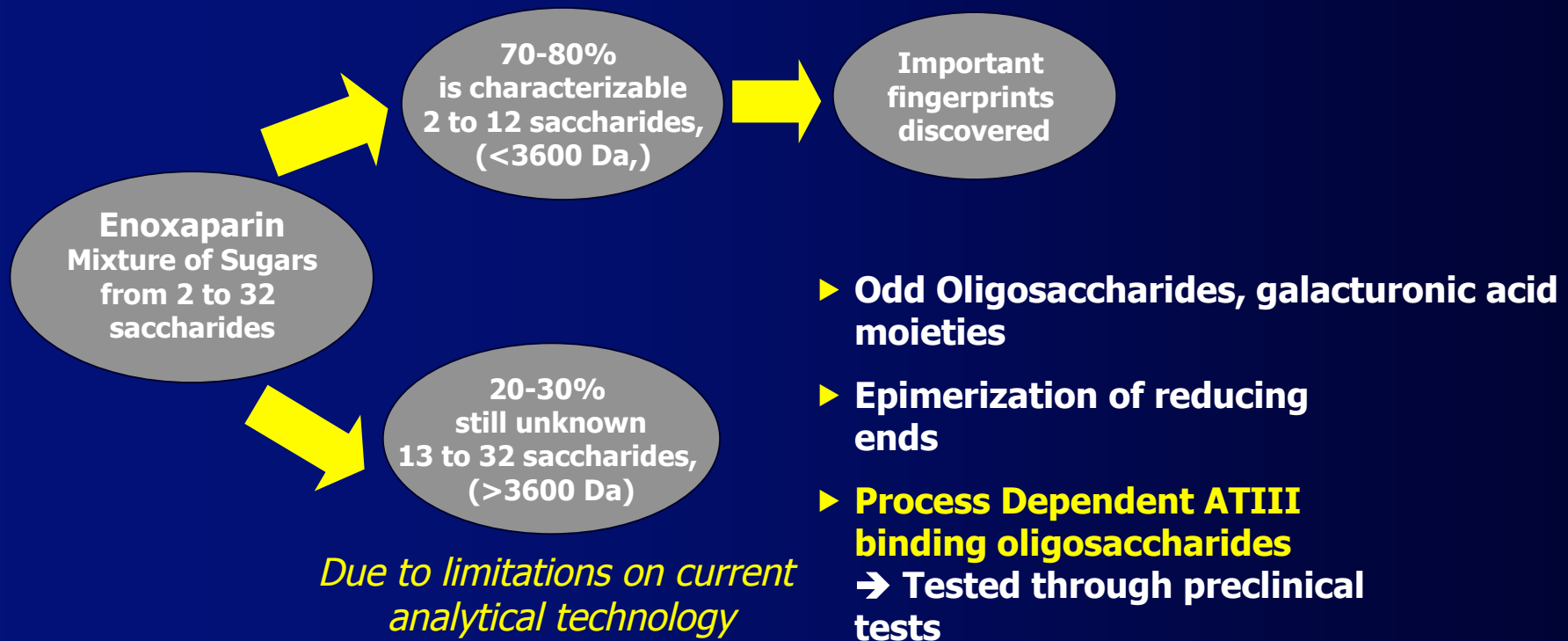
Trial	RRR	Thromboprophylaxis	Patients with VTE (%)
PRIME ¹ <i>P</i> <0.001 for equivalence	86%	UFH (5,000 IU t.i.d.) Enox. (40 mg q.d.)	 1.4* (n=443) 0.2 (n=442)
THE-PRINCE ² <i>P</i> =0.015 for equivalence	19%	UFH (5,000 IU, t.i.d.) Enox. (40 mg q.d.)	 10.4 [†] (n=212) 8.4 (n=339)
Hillbom, et al. ³ <i>P</i> =0.044	43%	UFH (5,000 IU, t.i.d.) Enox. (40 mg q.d.)	 34.7 [†] (n=72) 19.7 (n=76)

*VTE at day 7; [†]VTE at day 10.

1. Lechler E, et al. *Haemostasis*. 1996;26 Suppl 2:49-56
2. Kleber FX, et al. *Am Heart J*. 2003;145:614-21
3. Hillbom M, et al. *Acta Neurol Scand*. 2002;106:84-92

What has Been and What has Not Been Characterized in Enoxaparin ?

- ▶ The particular depolymerization process that results in enoxaparin leads to unique and highly complex mixture of oligosaccharide chains with different lengths, sequences & fingerprints



New Scientific Data Show How Previously Unknown Fingerprints Could be Pharmacologically Significant

Products tested [1.6 Anhydro Concentration]	1.6 Anhydro ring					AT III binding sequence
	Anticoagulation (Venous & Arterial)	Inflam. Nitric Oxid (ACS)	SMC proliferation (Restenosis)	Angiogenesis (STEMI/NSTEMI)	PK	Anticoagulation ATIII affinity
LMWH [7%], Enox [15%-25%] LMWH [40-50%]	Xa & IIa (high dose) TFPI Prothrombin Activation	+	+	FGF2 +	+	
Octa saccharides	TFPI Prothrombin Activation				Octa	+

Depending on the UFH Cleavage, Octasaccharides May Have Similar Anti-Xa Activity but with Widely Differing AT-III Affinity

Compound	Dissociation Constant (Kd in nM) 1 st series	Dissociation Constant (Kd in nM) 2 nd series	Anti-Xa Activity %*
Octa Δ I <u>I</u> a-II <u>s</u> -Is-Is	34.1 \pm 58%	13 \pm 70%	133
Octa Δ Is-IIa-II <u>s</u> -Is	334.0 \pm 82%	120 \pm 18%**	113
Penta SR90107/ORG31540	19.0 \pm 57%	20 \pm 39%	100

* Results normalized on pentasaccharide.

** Measurement repeated four times.

Summary

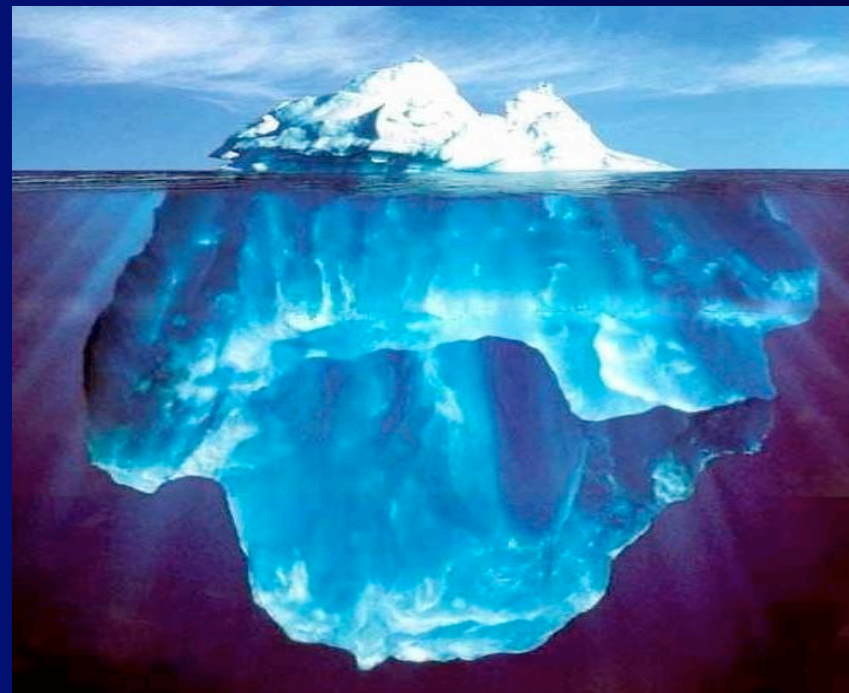
Technological
Limits
Oligosaccharides
< 13 sugars



Product
Characterizable
70% of compound



Specifications
Monograph & CEP
Anti Xa/IIa ratio
1.6 anhydro []
Octa Δ
(not yet)
Anti III binding sites



Oligosaccharides
> 13 sugars



30% of compound



Identified & Unknown
fingerprints
on Ols >13

Summary

- ▶ **The production of enoxaparin uses a tightly controlled manufacturing process that creates a complex collection of polysaccharides with a unique chemical structure**
- ▶ **This structure is characterized by distinct fingerprints that are highly sensitive to the manufacturing process**
- ▶ **Testing of 2 of these fingerprints found that they contribute to enoxaparin's pharmacological profile. Other known and/or yet-to-be discovered fingerprints may also contribute.**

Summary

- ▶ **Enoxaparin fits with the characteristics of a biological product given in amended Annex I to Directive 2001/83/EC**
- ▶ **In light of these data and in accordance with European Directives, enoxaparin biosimilarity cannot solely rely on duplication of MW, anti-Xa activity, and anti-Xa/anti-IIa ratio**
- ▶ **Biosimilarity should be established through exact sameness of manufacturing process or, failing that, through careful demonstration of similar and acceptable clinical outcomes**