Will plasma products inevitably be replaced by a new generation of therapeutics?

Dr. Jean-Francois PROST, Sr VP Scientific & Medical Affairs, LFB
Cyprus, May 9th, 2011
Historical ever-recurring question

Answers evolve with time, depending on various factors

A tentative to refresh the traditional debate

- global **scientific & medical view point** (i.e. not focused on manufacturing)
- aiming to **illustrate** instead of solving the question
- scope limited to **therapeutic proteins** (i.e. no ref. to advanced therapies)
Among «fractionators», recombinant products have already reached a significant market share.

### Therapeutic proteins as a whole

**Annual products market shares = 120 $Bn**
(sourced from Lehman Brothers)

- Alfa Interferon: 4%
- Beta Interferon: 9%
- Various: 2%
- EPO: 11%
- Insulin: 4%
- G-CSF: 1%
- Growth hormones: 2%
- FHS: 2%
- Monoclonal antibodies: 30%
- Plasma proteins: 12%
- Recombinant coagulation factors: 5%

### Plasma derived + recombinants

**Annual products turnover, $bn**
(sourced from MRB)

**Growth 1998-2008**

<table>
<thead>
<tr>
<th>Product Type</th>
<th>1998 Value ($Bn)</th>
<th>2008 Value ($Bn)</th>
<th>Growth 1998-2008</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>6.3</td>
<td>17.4</td>
<td>280%</td>
</tr>
<tr>
<td>Plasma</td>
<td>5.1</td>
<td>11.8</td>
<td>230%</td>
</tr>
<tr>
<td>Recombinant</td>
<td>1.2</td>
<td>5.6</td>
<td>460%</td>
</tr>
<tr>
<td>Market share</td>
<td>81%</td>
<td>69%</td>
<td></td>
</tr>
<tr>
<td>Market share</td>
<td>19%</td>
<td>31%</td>
<td></td>
</tr>
</tbody>
</table>

**Plasma**

- Value ($Bn): 5.1 in 1998, 11.8 in 2008 (230% growth)
- Market share: 81% in 1998, 69% in 2008

**Recombinant**

- Value ($Bn): 1.2 in 1998, 5.6 in 2008 (460% growth)
- Market share: 19% in 1998, 31% in 2008
**Most marketed plasma-derived proteins have their recombinant counterpart on the market or under dvpt**

<table>
<thead>
<tr>
<th>Reference protein</th>
<th>Plasma-derived product</th>
<th>Market value 2008 (MRB, $Bn)</th>
<th>Recombinant counterpart commercialized or in development</th>
<th>Market value 2008 (MRB, $Bn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII</td>
<td>Factane, Koate, Monoclate HemofilM, Octanate, Beriate Fanhdi, Alphanate, Profilate Factor 8Y, Replenate</td>
<td>1.5</td>
<td>Advate, Kogenate, Recombinate Xyntha, Helixate</td>
<td>4.0</td>
</tr>
<tr>
<td>FIX</td>
<td>Betafact, Octanine, Berinin Immunine, AlphaNine, Replene Mononine</td>
<td>0.3</td>
<td>Benefix</td>
<td>0.5</td>
</tr>
<tr>
<td>FVII</td>
<td></td>
<td></td>
<td>Novoseven</td>
<td>1.1</td>
</tr>
<tr>
<td>C-protein/ PPSB</td>
<td>Protexel, Ceprotin Kanokad</td>
<td>0.2</td>
<td>Xigris (activated)</td>
<td>0.4</td>
</tr>
<tr>
<td>Anti-thrombin</td>
<td>Aclotine, Ambinex Kybernin P, Thrombate III</td>
<td>0.3</td>
<td>Atryn</td>
<td>-</td>
</tr>
<tr>
<td>C1-inhibitor</td>
<td>Berinert, Cinryze</td>
<td>0.1</td>
<td>Ruconest (ex Rhucin)</td>
<td>-</td>
</tr>
<tr>
<td>FXIII</td>
<td>Fibrogammin-P</td>
<td>-</td>
<td>NoNovo-Nordisk (BLA)</td>
<td>Baxter (Phase III)</td>
</tr>
<tr>
<td>vWF</td>
<td>Willfact, Wilate, Hemate-P</td>
<td>0.3</td>
<td>Baxter (Phase III)</td>
<td>0.5</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Clottafact Riastap / Haemocomplettan</td>
<td>0.4</td>
<td>Profibrix, Pharming (pre-clinical)</td>
<td></td>
</tr>
<tr>
<td>AAT</td>
<td>Alfalastin, Zemaira, Glassia Prolastin, Aralast</td>
<td>0.4</td>
<td>GTC (pre-clinical)</td>
<td></td>
</tr>
<tr>
<td>FXI</td>
<td>Hemoleven</td>
<td></td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>Vialebex, Ydralbum Albunorm, Flexbumin</td>
<td>1.7</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>IVIg</td>
<td>Tegeline, Clairyg Kiovig/ Gammagard liquid Gammagard SD, Privigen Novagam, Octagam, Gampaglex Intratpect, Gamunex, Flebogamma DIF Carimune/ Sandoglobulin Ig Vena, Vigam</td>
<td>5.1</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>IGsc</td>
<td>Subcuvia, Hizentra Vivaglobin, Gammanorm, Subgam</td>
<td>0.1</td>
<td>-----</td>
<td></td>
</tr>
</tbody>
</table>
Among «fractionators», recombinant products occupy a dominant position in R&D pipelines.

- Overall R&D pipeline in accordance with cumulated turnovers
- Dominance of anti-hemophiliac factors (8 FVII, 7 FVIII, …)
- Emergence of mAbs
Plasma-derived vs recombinant proteins: Comparative arguments for starting a new dvpt

<table>
<thead>
<tr>
<th>Drivers</th>
<th>Recombinant</th>
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<tbody>
<tr>
<td>• Valorization of plasma supply</td>
<td></td>
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<tr>
<td>▪ Additional indications</td>
<td></td>
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<tr>
<td>▪ New products</td>
<td></td>
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<tr>
<td>• Natural products</td>
<td></td>
</tr>
<tr>
<td>▪ Low immunogenicity</td>
<td></td>
</tr>
<tr>
<td>▪ High bioavailability</td>
<td></td>
</tr>
<tr>
<td>• Limited regulatory burden</td>
<td></td>
</tr>
<tr>
<td>• Protein structure under control (sequence, ± PTM)</td>
<td></td>
</tr>
<tr>
<td>• High IP potential</td>
<td></td>
</tr>
<tr>
<td>• Innovative entities with original pharmacodynamic profile</td>
<td></td>
</tr>
<tr>
<td>• Access to therapeutic areas beyond traditional plasma franchises</td>
<td></td>
</tr>
<tr>
<td>• Biological safety</td>
<td></td>
</tr>
<tr>
<td>• Production capabilities</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Brakes</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>• Biological safety perception</td>
<td></td>
</tr>
<tr>
<td>• Restricted diversity of proteins</td>
<td></td>
</tr>
<tr>
<td>• Limited supply</td>
<td></td>
</tr>
<tr>
<td>• Post-Translational Modifications mastering (depending on expression system)</td>
<td></td>
</tr>
<tr>
<td>• Complex regulatory path</td>
<td></td>
</tr>
<tr>
<td>• High CaPex</td>
<td></td>
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</tbody>
</table>
Biological safety

Still a momentum for moving from plasma to recombinant products?
Classical transfusion viral risks have become extremely low

Residual risk (RR) of transmission of viral infections per million donations

Exhaustive epidemiological register of all French blood donors and donations 1992-2009

(1 700 000 donors, 3 200 000 donations in 2009)

<table>
<thead>
<tr>
<th>Taux incidence / 10^5 P-A (IC 95%)</th>
<th>Risque résiduel (IC 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIH</td>
<td>1.01 p.10^5 (0.67 - 1.50)</td>
</tr>
<tr>
<td>HTLV</td>
<td>0.12 p.10^5 (0.03 – 0.37)</td>
</tr>
<tr>
<td>VHC</td>
<td>0.35 p.10^5 (0.17 – 0.69)</td>
</tr>
<tr>
<td>VHB*</td>
<td>0.90 p.10^5 (0.58 – 1.36)</td>
</tr>
</tbody>
</table>

1992-99 : RR dramatic decline
- serology on mini-pools
- donors selection
- lower incidence of the infections

2000- : RR additional reduction
- Nucleid acid detection (PCR) on HIV-1 & HCV
- Secondary reduction expected with introduction of VHB PCR

*données ajustées pour tenir compte du caractère transitoire de l’Ag HBs
Grifols press release: a new treatment!

Grifols receives FDA approval for Alphanate® to treat vCJD
Written on March 5, 2011

New preventive therapies of prion neurotoxicity (aspirin !)

Prion peptide-mediated cellular prion protein overexpression and neuronal cell death can be blocked by aspirin treatment.
Jeong JK, Moon MH, Seol JW, Seo JS, Lee YJ, Park SY.
Chonbuk National University, Jeonju, Republic of Korea.

Or of prion intestinal invasion (amino-caproïc acid !)

Blocking of FcR Suppresses the Intestinal Invasion of Scrapie Agents.
University of Tokyo, Tokyo, Japan.
Despite several persistent uncertainties…

- **Still new cases in the UK**
  - Considering the possibility of a second wave of epidemics in “non MM patients”?

- **Precise nature of prion in blood unknown**
  - Challenging the pertinence of validation methods (spikes) for safety steps in manufacturing processes

- **Atypical delayed BSE forms in transmission experiments in macaques (Comoy, *et al.*, Prion 2010).protobuf
  - Questioning about the real nature (additional to PrPres?) and pathogenic dose (low?) of the infectious agent

- **“English haemophiliac patient”**
  - Raising concerns about the actual exposure of hemophiliacs through low purity FVIII concentrates extracted from non-leucodepleted plasmas

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**Haemophilia**

**ORIGINAL ARTICLE**

Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia

- 1 positive spleen sample out of 26 tested (WB)
Prion-related risk appears under control

1. Confirmed overall decline of vCJD epidemics

### Countries and Case Numbers

<table>
<thead>
<tr>
<th>Country</th>
<th>Case numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK</td>
<td>172+3*</td>
</tr>
<tr>
<td>France</td>
<td>25</td>
</tr>
<tr>
<td>Irlande</td>
<td>4</td>
</tr>
<tr>
<td>Italy</td>
<td>2</td>
</tr>
<tr>
<td>USA</td>
<td>3</td>
</tr>
<tr>
<td>Canada</td>
<td>2</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>1</td>
</tr>
<tr>
<td>Japan</td>
<td>1</td>
</tr>
<tr>
<td>Netherlands</td>
<td>3</td>
</tr>
<tr>
<td>Portugal</td>
<td>2</td>
</tr>
<tr>
<td>Spain</td>
<td>5</td>
</tr>
<tr>
<td>Taiwan</td>
<td>1</td>
</tr>
</tbody>
</table>

*Transmission by transfusion*
Prion-related risk appears under control

2-Recognized efficacy of securitization processes

- Efficacy statements introduced in regulatory guidelines and recent labeling

“Available data indicated that the manufacturing processes in plasma-derived medicinal products would reduce vCJD infectivity if it were present in human plasma.”

(EMA- CHMP position statement on CJD and plasma-derived medicinal products, 2004)

“Manufacturing steps adopted by the company provide reasonable assurance that low levels of CJD/vCJD agent infectivity, if present in the starting material, would be removed.”

(FDA-approved revised labeling of Alphanate)

- New affinity removal processes under development

.../...
Prion-related risk appears under control

3 - Emergence of new detection methods

Capture: 
- Extract and enrich the signal.
- Allows to bypass the inhibitory effect of plasma.
  § Affinity ligands (plasminogen, PRDT, LDLs)
  § Metals
  § Magnetic Beads (Nanoparticles)

Amplification: 
- Compensates for the extremely low infectivity level in plasma (10 à 20 UI/ml).
- Minimal amplification required for detection estimated to be >10 (plasma pools).
  § Protein Misfolding Cyclic Amplification (PMCA) - Sonication
  § Quaking Induced Conversion (QuIC)
  Quaking (agitation)

Revelation: 
- Detection of the different PrP TSE forms (RES, SEN)
  § Western Blot (WB)
  § Conformation Dependent Immunoassay (CDI)
  § Bioassay

The prion protein detectable in CSF of vCJD patients (specificity 100 %, sensitivity 85 %)
Atarashi et al. Nat Met 2011

A new method based on the capture of prions from plasma allows for the detection of vCJD patients
Collinge et al, Lancet 2011

A second capture method, allows for the removal of prions from solutions
Miller et al, 2011 JVI.
The risk of variant Creutzfeldt-Jakob disease among UK patients with bleeding disorders, known to have received potentially contaminated plasma products

N=787
604 followed up > 13 y

patients had developed clinical vCJD. The absence of clinical vCJD cases in this cohort to date suggests that either plasma fraction infectivity estimates are overly precautionary, or the incubation period is longer for this cohort than for implicated cellular blood product recipients. Further follow-up of this cohort is needed.
Biological safety

Polyvalent immunoglobulins

*May not remain the “traditional sanctuary” of plasma derivatives*?
While being a WHO essential therapy in PID, IVIg have been increasingly used in AIDs.

WW IgIV sales (tons)
source: MRB

CAGR +8%

Auto-Immune Diseases

Immune deficiencies
Multiple immunomodulating MoA would make IVIg “irreplaceable/invulnerable” in AIDs

Interaction with soluble or cell membrane molecules involved in pro-inflammatory phenomena

Prevention of effector cells activation (ITAM, ITIM) and accelerated clearance of auto-antibodies (FcRn)

Repertoire diversity: Fab’(2) dependent

Effector functions: Fc-dependent

Multiple immunomodulating MoA would make IVIg “irreplaceable/invulnerable” in AIDs

- \(\alpha\)CD20
- \(\alpha\)IL6-R
- \(\alpha\)CD40
- \(\alpha\)LFA-1
- \(\alpha\)CD3
- \(\alpha\)IL-1
- Recombinant Fc
- \(\alpha\)TNF
- \(\alpha\)IL1
- \(\alpha\)IL1-soluble R
- \(\alpha\)C5a
- \(\alpha\)CD32 b
- \(\alpha\)CD16
IVIg are increasingly studied in AIDs

Number of publications related to clinical trials in AIDs (n=267) over the last 10 years as a function of the disease incidence.

Beyond core SPC, IVIg are on the way for regulatory validation in niche indications i.e. Myasthenia, Multifocal Neuropathy and Demyelinating Polyneuropathy (CIDP)

Source: LFB scientific survey (Medline)
IVIg are challenged by “versatile-target” Mabs (anti-CD20)

⇒ Although CD20 mAbs were mainly focused on larger indications (RA, MS, Lupus), they progressively penetrate IVIg niche “territory”
Jones RB., NEJM, 2010
Rituximab versus cyclophosphamide in ANCA-associated renal vasculitis.

Hauser SL., NEJM, 2008
B-cell Depletion with rituximab in relapsing-remitting Multiple Sclerosis.

Joly P., NEJM, 2007
A single cycle of rituximab for treatment of severe pemphigus.

Ramos-Casals M., Clin Exp Rheumatol, 2010
Off-label use of rituximab in 196 patients with severe, refractory systemic AID.

-> BLA submitted in Nov 2010 to FDA
The increasing competition forces fractionators
to implement \textit{incremental} improvements:

- \texttt{Active} regulatory validation of current niche indications
- New formulations and routes of administration (SC)
- Continuous improvement of extraction yield

\texttt{to explore \textit{disruptive} approaches}

- Sub-fractionation of the IVIg “soup” (Pr. SHOENFELD)
- Structural IVIg optimization by post-process modifications (Pr. Ravetch)
- \texttt{Investigation of innovative clinical avenues}
Innovative clinical avenues
1– Severe sepsis

Two mechanisms of action of IVIg
- immuno-modulation ➔ over stimulation of acute phase
- immuno-substitution ➔ suppression of post-acute phase

ADULTS
Sepsis: rethinking the approach to clinical research

NEONATES
Study protocol
The INIS Study,
International Neonatal Immunotherapy Study
intravenous immunoglobulin therapy
for suspected or proven neonatal sepsis
https://www.npeu.ox.ac.uk/inis

3493 babies
between Oct 2001 and Sept 2007
across 9 different countries
Up to 2-year follow-up
Study completed
Results expected shortly

Anti-endotoxin Therapy
9 trials; 3057 Patients

Anti-TNF Antibodies
10 Trials; 6821 Patients

IL-1ra
3 Trials; 1688 Patients

Intravenous immune globulin
20 Trials; 2821 Patients

Activated Protein C; All Patients
2 Trials; 4303 Patients

Activated Protein C; Patients with MOF
2 Trials; 2133 Patients

Experimental Agent Better
Placebo Better
Human intravenous immunoglobulin provides protection against Aβ toxicity by multiple mechanisms in a mouse model of Alzheimer’s disease.

Dose-dependant effect of IVIg on hippocampal neurons

Abeta neuro-toxicity

Dose-dependant effect of IVIg on Abeta deposits clearance

Abeta deposits co-localization with microglia
Clinical avenues 2 – Alzheimer’s disease clinical trials

Phase II (completed, n=24)  
(N. Relkin communication, IPPC, Lisbon, March 2011)
- 6 mo. placebo-controlled, followed by 12 mo. open-label treatment
- Improvement of cognition functions (ADAS-Cog)
- Correlated with reduction of ventricular enlargement @ 18 mo.
- Better dose: 0.4 g/kg/ 2wk (n=4) compared to 0.4 g/kg/ 4wk and 0.8 g/kg/ 4wk

Phase III (Baxter study ongoing, n=360, 44 sites)
- 18 mo. placebo-controlled, randomized study (200 and 400 mg/kg every 2 weeks)
- FPI: Dec. 2008
- Final data collection date for primary outcome measure: July 2011

Potential consequences for plasma supply if approved in AD (MRB, 2009)
- Plasma demand per patient: 100-300 liters/year
- Increase in plasma collection and process: + 39 Mo. liters/year
IVIg: the future...

The increasing competition forces fractionators

- To implement incremental improvements
- To explore disruptive approaches
- ...and to **diversify to mAbs**

Source: DATAMONITOR – October 2009
The anti-RhD case study

Hyperimmune polyvalent Ig can be replaced by “specific-target” antibodies

## Rhesus incompatibility
- Immunization of Rh- mothers against the Red Blood Cells (RBC) of their Rh+ fetus ➔ *600 000 concerned pregnancies per year in Europe*
- Not prevented, Rh- immunization is a major cause of spontaneous abortion or fetal malformation during subsequent pregnancies ➔ *immunization rate = 16%*
- Prophylaxis applied using pre-/post-natal anti-D polyvalent Ig ➔ *immunization rate reduced to 0.35%*

## Remaining issues
- Polyvalent immunoglobulin preparations requires Rh- healthy volunteers immunization (banned procedure in most European countries)
- Safety concerns associated with blood product administration in pregnant women ➔ *in France, remaining 750 cases of allo-immunization per year (50 to 100 deaths)*

Need for a worldwide prophylaxis treatment of Rh incompatibility devoid of ethical issues manufacturing constraints.
Roledumab (LFB-R593), a mAb dedicated to anti-RhD prophylaxis

Pre-clinical: a biological profile meeting the therapeutic requirements

- Fully human antibody
- Recognition rate of RhD+ RBC = 99.96% (50,000 tested sera)
- Glyco-engineered antibody, with high affinity for CD16 and high ADCC on RhD+ RBC leading to RBC phagocytosis

![Graph showing % RBC lysis vs Ab ng/ml (LOG)]

- Polyclonal
- negatif control
- pilot batch of LFB-R593
- clinical batch of LFB-R593
Roledumab (LFB-R593), a mAb dedicated to anti-RhD prophylaxis

**Phase II: a high efficiency on RhD+RBC clearance, following IV & IM routes (N=78)**

[Graphs showing RhD-positive RBC clearance over time for IV and IM routes with different doses of LFB-R593 and Rhophylac]
Biological safety

Polyvalent immunoglobulins

Coagulation factors

**Inhibitors issue remains pending**

**Time has already come for innovative molecular entities**
F VIII inhibitors: 2 Meta-analyses in PUPs

<table>
<thead>
<tr>
<th></th>
<th>Iorio et al, 2010</th>
<th>Franchini et al, 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td># studies</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td># patients total</td>
<td>2094</td>
<td>800</td>
</tr>
<tr>
<td># patients pd-FVIII</td>
<td>1167</td>
<td>363</td>
</tr>
<tr>
<td># patients rec-FVIII</td>
<td>927</td>
<td>437</td>
</tr>
</tbody>
</table>

Quality review of the studies

- MOOSE\(^1\) + STROBE\(^2\) guidelines
- NOS\(^3\) Scale + STROBE\(^2\) guidelines

Incidence rate of inhibitors [95% CI]

- pd-FVIII: 14% [10-19]
- rec-FVIII: 27% [23-31%]

P value

- <0.001
- NS

High responders (Inhibitors > 5 UB)

- pd-FVIII: 9% [6-13%]
- rec-FVIII: 17% [14-21%]

Influencing factors on inhibitors titers

- recency of study period follow-up duration testing frequency
- prophylaxis

1. MOOSE : Meta-analysis Of Observational Studies in Epidemiology
2. STROBE : Strengthening The Reporting of Observational Studies in Epidemiology
3. NOS : Newcastle Ottawa Scale
European Haemophilia Safety Surveillance System (EUHASS)

Post-marketing International AE reporting system

- 15,030 patients with Haemophilia A & B surveyed between Oct 2009 & Sept 2010, including 153 PUPs
- 64 centres in 27 countries

**Incidence rate**

- r-FVIII: 25% [19–33]  N=35/138

SIPPET (Survey of Inhibitors in Plasma-Product Exposed Toddlers)

- 300 patients overall, i.e. 150 per arm
- 30 active centres (India, Egypt, EU, South Africa & US)
- Hypothesis: ∆ 50% of the cumulative incidence of inhibitors for VWF/FVIII vs rFVIII (30%) ; 1-β=0.80, α=0.05
- FPI: Oct 2010, 150 patients included, 1st interim report (futility analysis): mid-2012
Site-directed PEGylation [1]

Polyethylene glycol polymer conjugated to surface-exposed cysteines introduced to FVIII variants (Bayer) to preserve activity

Improved pharmacokinetics in hemophilic mice (5.9 vs 9.8 hrs) and rabbits (6.7 vs 12 hrs)

Prolonged efficacy in bleeding models of hemophilic mice
Recombinant factor IX-Fc fusion protein (rFIXFc) contains a single molecule of FIX recombinant attached to the constant region (Fc) of immunoglobulin G (IgG)

Dramatically improved pharmacokinetics with effective blood levels being present at the 4th day in mice and persisting to 1 week in dog

Completed rFIXFc Ph I/IIa trial and transition to global pivotal licensure studies
Potential issues associated with new entities

- **Manufacturing**
  - Cell line production yield
  - Industrial scale-up (PEGylation)
  - Increased CoG

- **Clinical tolerability**
  - Potential increased immunogenicity (mutated entities)
  - Non-specific toxicity of New Biological Entities (highly PEGylated entities)
  - Thrombogenic potential (?)

- **Pharmacodynamics**
  - Product-related specific coagulant activity assessment vs medical culture of biological monitoring
  - Concurrent loss of intrinsic efficacy (highly PEGylated entities)

➤ Likely to be overcome
Biological safety

Polyvalent immunoglobulins

Coagulation factors

Other classical plasma proteins

*Disillusion or field of hope?*
Supplementation in Disseminated Intravascular Coagulation (DIC) has been unsuccessful

- **Anti-thrombin**
  - Plasma-derived AT (Aclotide®, Thrombate®, …) : approved in Inherited Deficiency (ID)
  - Kibersept® study in DIC associated sepsis: unconclusive results (Warren, JAMA, 2001)
  - Atryn® : Phase II interrupted (study conduct issues)

- **C Protein**
  - Plasma-derived C protein (Ceprotein®, Protexel®) : approved in ID and purpura fulminans
  - Activated recombinant C protein (Xigris®) : approved in sepsis but with limited benefit

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**Marti-Carvajal AJ et al.**
Human recombinant activated protein C for severe sepsis.
Cochrane Database Syst Rev. 2011 Apr 13;4

- **Hospital Mortality** : RR 1.01 [0.87-1.16] NS
- **Bleeding** : RR 1.47 [1.09-2.0] p = 0.01
Other classical plasma-derived proteins represent a significant growth reservoir

- **Alpha 1-Antitrypsin**
  - Approved in Deficient Emphysema on Phase II PK studies basis
  - Recent (Nov 2010) combined analysis of 2 Phase II studies with ALFALASTIN® and PROLASTIN® showed significant reduction in the decline lung density (CT scan)
  - Phase III/IV studies with PROLASTIN® ongoing (n=180 patients treated for 2 years + follow-up)
  - Inhaled alpha 1-antitrypsin (Kamada) : Phase II/III study ongoing (n=200)

- **Fibrinogen**
  - Approved in inherited deficiency (RIASTAP®, CLOTTAFACT®)
  - Planned studies in serious bleedings associated with blood dilution coagulopathy: trauma, surgery and post-partum haemorrhages

- **Albumin**
  - Well-recognized effect on morbidity/mortality in complicated cirrhosis
  - Completed trial in severe sepsis: EARSS, n=794 in 15 centres (LFB)
  - Ongoing trial in acute ischemic stroke: ALIAS, n=1100 in 25 centres (NINDS)
Albumin corrects hypoalbuminemia (EARSS study, n=794 - JP Mira, ISICEM, 2011)

**Survival**

- Albumin group
- Control group

**Mortality:**
- Albumin: 24.1% Control group: 26.3% (NS)
- Absolute mortality reduction: 2.2%
- Relative mortality reduction: 8.4%

**% of patients with Albuminemia < 25 g/L**

<table>
<thead>
<tr>
<th></th>
<th>H-12</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin group</td>
<td>88</td>
<td>60</td>
<td>38</td>
<td>23</td>
<td>36</td>
</tr>
<tr>
<td>Control group</td>
<td>88</td>
<td>91</td>
<td>93</td>
<td>93</td>
<td>93</td>
</tr>
</tbody>
</table>

**Albuminemia**

- Days: 0, 5, 10, 15, 20, 25
- g/L: 0.4, 0.6, 0.8, 1.0

* indicates statistical significance.
As a consequence, the outcome appears interestingly in favor of plasma derivatives

**Current conservative scenario**
- Plasma fractionators “satisfied” with moderate sales growth relying on a fragile regulatory basis
- New recombinant entrants still discouraged by
  - “50/50” results of plasma derivatives
  - heavy burden of the clinical proof

**Mid-term expansive scenario**
- Plasma derivatives (Fibrinogen, Alpha 1-Antitrypsin) regulatorily validated in their major indications (a few years further dvpt)
- Recombinants tempted to mimic and follow up this success, but confronted with:
  - complex manufacturing issues (FG, AAT)
  - low pricing (Albumin)

**In the long term**
- Potential opportunities for second-generation products (cf anti-haemophiliacs)
Biological safety

Polyvalent immunoglobulins

Coagulation factors

Other established plasma proteins

New proteins

*Human plasma, still a wise source?*
Search for new plasma-derived proteins can be viewed as an economic-wise approach

- Valorization of fractionated plasma in addition to the major volume products (IVIg, albumin, FVIII and AAT)
- Valorization of under-exploited intermediates or fractions
- Opportunity to mutualize under-loaded equipment
- Potential of introducing new processes for new products within the current fractionation tree with limited re-validations
The plasma proteome displays high diversity and multiple functions

- **High structural diversity**
  - 500 « true » plasma proteins, most of them glycosylated
  - ~ 20 different glycosylated isoforms per protein
  - ~ 5 different apparented entities (variants, precursors, truncated)

- **Variable abundance**
  - 10 orders for magnitude from 35-50 g/l (albumin) to < 1 pg/ml

- **Multiple functions**
  - Blood coagulation & fibrinolysis
  - Complement system
  - Immune system
  - Enzymes
  - Inhibitors
  - Lipoproteins
  - Hormones
  - Cytokines and growth factors
  - Transport and storage

---

www.sigmaaldrich.com, adapted from Putnam 1975-1987
Search for new plasma-derived proteins should meet appropriate conditions

**OPTIMALLY:**

- Physiological presence in a significant concentration in plasma (≈ 0.5 mg/ml) vs the expected therapeutic dose

- Recognized pathology related with inherited/acquired deficiency of the protein (Nature provided PoC)

- Serious expected production issues with recombinant technologies for the protein of interest (complexity, size ...)

- Potential for being a “surrogate” product for a rapid PoC

**ALTERNATIVELY**

- Potential for a specific and effective extraction process

- Critical role in the pathophysiology of a well-defined disease (“drugability”)

- No recombinant “challenger” foreseen in the near future

- In anticipation of the fully developed recombinant version
Most “classical” pertinent plasma proteins have already been explored

<table>
<thead>
<tr>
<th>Highly Abundant &gt;1000 mg/l</th>
<th>Moderately Abundant 100-1000 mg/l</th>
<th>Low Abundant 1-100 mg/l</th>
<th>Very Low &lt; 1 mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Blood coagulation &amp; fibrinolysis</td>
<td><strong>Fibrinogen</strong></td>
<td>beta glycoproteins</td>
<td>Pre-Kallikrein</td>
</tr>
<tr>
<td></td>
<td>Vitronectin</td>
<td>F XIII</td>
<td>FV</td>
</tr>
<tr>
<td></td>
<td>Fibronectin</td>
<td>F X</td>
<td>FXI</td>
</tr>
<tr>
<td></td>
<td>Plasminogen</td>
<td>Protein S</td>
<td>FIX</td>
</tr>
<tr>
<td></td>
<td>Kininogen</td>
<td>UPA</td>
<td>TAFI</td>
</tr>
<tr>
<td></td>
<td>Thrombospondin</td>
<td>Tetranectin</td>
<td>Protein C</td>
</tr>
<tr>
<td></td>
<td>Prothrombin</td>
<td>FvW</td>
<td>FXII</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FX</td>
<td>FVIII</td>
</tr>
<tr>
<td>2. Complement system</td>
<td>C3</td>
<td>C4</td>
<td>C5</td>
</tr>
<tr>
<td></td>
<td>Factor H</td>
<td>C8</td>
<td>Factor I</td>
</tr>
<tr>
<td></td>
<td>C4b BP</td>
<td>C9</td>
<td></td>
</tr>
<tr>
<td>3. Immune system</td>
<td>IgG</td>
<td>alpha-1 acid glycoprotein</td>
<td>IgD</td>
</tr>
<tr>
<td></td>
<td>IgA</td>
<td>alpha-2 glycoprotein</td>
<td>alpha microglobulin</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
<td>Zinc alpha2 glycoprotein</td>
<td>beta 2 microglobulin</td>
</tr>
<tr>
<td>4. Enzymes</td>
<td>Angiotensin Converting Enzyme</td>
<td>Arylesterase</td>
<td>Lysozyme</td>
</tr>
<tr>
<td></td>
<td>Carboxypeptidase</td>
<td>Glutathion peroxydase</td>
<td>Cholinesterase</td>
</tr>
<tr>
<td>5. Inhibitors</td>
<td>AAT</td>
<td>ITI</td>
<td>Heparin cofactor 2</td>
</tr>
<tr>
<td></td>
<td>alpha-2 macroglobulin</td>
<td>anti-Chymotrypsin</td>
<td>Anti-plasmin</td>
</tr>
<tr>
<td></td>
<td>AT</td>
<td>AT</td>
<td>Kallistatin</td>
</tr>
<tr>
<td></td>
<td>C1 inhibitor</td>
<td>C1 inhibitor</td>
<td>PAI-3</td>
</tr>
<tr>
<td>6. Lipoproteins</td>
<td>Apo-A1</td>
<td>Apo-B</td>
<td>Apo-D</td>
</tr>
<tr>
<td></td>
<td>Apo-A2</td>
<td>Apo-Lp(a)</td>
<td>Apo-E</td>
</tr>
<tr>
<td></td>
<td>Apo-C3</td>
<td>Apo-C1</td>
<td>SAA</td>
</tr>
<tr>
<td></td>
<td>Apo-A4</td>
<td>Apo-C2</td>
<td>LCAT</td>
</tr>
<tr>
<td>7. Hormones</td>
<td>Angiotensinogen</td>
<td>Adiponectin</td>
<td></td>
</tr>
<tr>
<td>8. Cytokines &amp; growth factors</td>
<td></td>
<td></td>
<td>IL1a, IL-8, G-CSF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IFN-α, IL-2, IL-4</td>
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<tr>
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<td>TNF-α, IFN-γ, IFN-β</td>
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<td>IL-12, IL-5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IL-10, IL-6</td>
</tr>
<tr>
<td>9. Transport &amp; storage</td>
<td><strong>Albumin</strong></td>
<td>Hémöpectin</td>
<td>Transcoritin</td>
</tr>
<tr>
<td></td>
<td>Transferrin</td>
<td>Vit.D binding protein</td>
<td>Retinal binding protein</td>
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<tr>
<td></td>
<td></td>
<td>Transerythrin</td>
<td>Selenoprotein P</td>
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<td></td>
<td></td>
<td>Gelsolin</td>
<td>ALS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>alpha 1 glycoprotein</td>
<td>Thyroxin Binding Protein</td>
</tr>
<tr>
<td></td>
<td>Haptoglobin</td>
<td>Ceruloplasmin</td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>10</td>
<td>32</td>
<td>58</td>
</tr>
</tbody>
</table>
New developed plasma proteins focus on niche indications and may already have their recombinant counterparts.

<table>
<thead>
<tr>
<th>New plasma protein in development</th>
<th>Company</th>
<th>Indication</th>
<th>Plasma product stage</th>
<th>Recombinant follow-up stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor X</td>
<td>BPL</td>
<td>FX deficiency</td>
<td><strong>Phase III</strong></td>
<td>-</td>
</tr>
<tr>
<td>Reconstituted HDL</td>
<td>CSL</td>
<td>Coronary atherosclerosis</td>
<td>CSL-111 <strong>Phase IIa</strong> CSL-112 <strong>Phase I</strong></td>
<td>r-ApoA1 Cerenis (CER-001) <strong>Phase II</strong> r-ApoA1 (ETC-216) <strong>Phase II</strong></td>
</tr>
<tr>
<td>C-SAP</td>
<td>LFB</td>
<td>Amyloidosis diagnosis</td>
<td>Clinical POC</td>
<td>-</td>
</tr>
<tr>
<td>Plasmin</td>
<td>Talecris</td>
<td>Direct-acting thrombolytic</td>
<td><strong>Phase I/II</strong></td>
<td>Talecris <strong>Pre-clinical</strong></td>
</tr>
<tr>
<td>Transferrin</td>
<td>Sanquin Kedrion Kamada</td>
<td>Tf deficiencies + Adjuvant cancer therapy</td>
<td><strong>Phase I/II</strong></td>
<td>-</td>
</tr>
<tr>
<td>Butyryl-cholinesterase</td>
<td>Baxter</td>
<td>Exposure to chemical nerve agent</td>
<td><strong>Phase I</strong></td>
<td>-</td>
</tr>
<tr>
<td>Interalpha trypsin inhibitor</td>
<td>Sanquin</td>
<td>Sepsis</td>
<td><strong>Pre-clinical</strong></td>
<td>-</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>Kedrion</td>
<td>Ligneous Conjunctivitis</td>
<td><strong>Pre-clinical</strong></td>
<td>-</td>
</tr>
<tr>
<td>Factor H</td>
<td>LFB Baxter CSL</td>
<td>Atypical Hemolytic Uremic Syndrome</td>
<td><strong>Pre-clinical</strong></td>
<td>anti-C5 (Eculizumab, Alexion) <strong>Marketed</strong></td>
</tr>
</tbody>
</table>
Concluding remarks

Will plasma products inevitably be replaced by a new generation of therapeutics??
CONCLUDING REMARKS (1)
Plasma business is subject to growing “recombinant pressure”

- **Despite undeniable assets**
  - Irreplaceable and essential therapy with IVIGs
  - Traditional safety issues now put under control

- **Serious concerns come from several factors**
  - Emergent *improved coagulation factors for Haemophilia* which will supplant classical plasma derived products
  - Progressive *penetration of IVIg niches in Auto-Immune Diseases by various specific Mabs*
  - Labelled, but *remaining clinically non-validated indications of “volume products”* such as Albumin, Fibrinogen, Alpha-1 Antitrypsin

- **Impacts of which aggravated by the manufacturing specificity of plasma fractionation**
  - Persistent need for amortizing plasma supply with several finished products in addition to IVIg

⇒ **Recombinants put at risk the whole fractionation tree**
CONCLUDING REMARKS (2)
Continuing to value plasma relies on a mix

Non-IGIVs, non anti-hemophiliac products

→ Conservative accessible priority:
  - Validate “third leg” products with powered trials in their labelling (AAT in emphysema, Fg in severe bleedings)

→ Innovative risky upsides:
  - Search for additional robust indications in abundant products (AAT in diabetes? in fibromyalgia ?, albumin in stroke ?)
  - Explore synergistic combinations (FVII+Fg, FXIII+Fg, AAT + C protein, …)
  - Develop new products from plasma in niche indications (ApoA1, plasmin, factor H, …)

→ Will those attempts be sufficient to ensure a sustained growth in the mid term?
CONCLUDING REMARKS (3)
Continuing to value plasma relies on a mix

IVIGs:

- **Like other products, prioritize conservative approaches**
  - Finalize IVIG validation in Ig-dependant auto-immune diseases to protect against “mAbs invasion”

- **As the plasma driver, lower the pressure on plasma demand**
  - Develop high yield processes and decrease CoGs
  - Allowing to access to major indications (AD, sepsis)

- **As the plasma driver, increase its intrinsic value**
  - Transform the “one-fits-all” product in a number of **sub-fractions** addressing selected autoimmune pathologies with significant increase in therapeutic index and dose reduction (<< 1 g/kg)

- **if AD validated, candidate indication for sub-fractionation strategy**
CONCLUDING REMARKS (4)
Plasma-associated expertise can be leveraged

In addition to plasma, fractionators could continue leveraging their protein expertise and severe diseases knowledge to diversify

- **In terms of products ➔ mAbs**
  - Structural plasticity and diversity of targets
  - Fast-growing markets

- **In terms of manufacturing technologies ➔ therapeutic transgenesis**
  - Major improvements in the production yield
  - Reduced and highly flexible capital expenditure
  - Ability to face cost-containment measures threatening biotech products

**➔ will animal milk donors replace human blood donors??**
In our ethical business, some wishful thinking ... 

- Develop a wise balanced competition?
  - 24 products in research for hemophilia ...
  - 97% off-label use for recombinant FVIIa ®

- Coordinate efforts in clinical development?
  - Multi-sponsored mega-trials for validation of classical products (SIPPET, INIS, antithrombins ?)
  - Parallelized pivotal studies in the same indication leading to results cross-validation (IVIG, Fibrinogen, …)

- Keep safety as the top priority!
  - Biological safety not only in plasma
  - Purity not only in biotech
Thank you!

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H. Thomas
R. Urbain

Hemostasis
Safety
IVIG & mAbs
R&D survey
Market data
All!