Development of monoclonal antibodies (mAb) in onco-hematology

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INTRODUCTION
Naked antibody structure (mammalian species except camelids)

Ribbon model of IgG1, yellow circles represent glycan structures

Binding Site: specificity and affinity

Fc region, effector function

Ribbon model of IgG1, yellow circles represent glycan structures
Approved monoclonal antibodies

**Naked cytotoxic**
- rituximab Mabthera /Rituxan CD20 chimeric 1997 Non Hodgkin Lymphoma
- trastuzumab Herceptin HER2 humanized 1998 Breast
- alemtuzumab MabCampath CD52 humanized 2001 Chronic lymphocytic Leukemia
- cetuximab Erbitux EGFR chimeric 2004 Colorectal Head and Neck
- panitumumab Vectibix EGFR human 2006 Colorectal
- ofatumumab Arzerra CD20 human 2009 Chronic lymphocytic Leukemia

**Naked blocking**
- bevacizumab Avastin VEGF humanized 2004 Colorectal

**Naked immunomodulating**
- Ipilimumab Yervoy CTLA4 human 2011 Melanoma

**Radio conjugated**
- ibritumomab Zevalin CD20 murine 2002 Non HodgkinLymphoma
- toxitumomab Bexxar CD20 murine 2003 Non Hodgkin Lymphoma (FDA)

**Drug Conjugated**
- brentuximab Adcetris CD30 human 2011 CD30+ lymphoma (FDA)

**Bi-specific**
- catumaxomab Removab Epcam murine/rat 2009 Malignant ascitis (EMA)
INTEREST AND LIMITATIONS OF HISTORICAL (CYTOTOXIC) mAbs
Interest of rituximab in Non Hodgkin Lymphoma (NHL)

- Example of previously untreated elderly patients (> 60 years) with large cell NHL
  - N = 399
  - rituximab-CHOP vs CHOP (cyclophosphamide, adriamycin, Oncovin, prednisone)

Results achieved in relapsed NHL

Cartron G et al Clin Cancer Res 2011; 17: 19-30
Success and limitations of rituximab

• Limited efficacy as monotherapy
  o Interest as maintenance therapy in Follicular Lymphoma
    • Salles G et al Lancet 2011; 377: 42

• Interest as combination with poly-chemotherapy
  o Non Hodgkin Lymphoma: 10% improvement in survival at 4-5 years
    • Diffuse Large B Cell Lymphoma (R-CHOP)
    • Follicular lymphoma (R-various polychemo)
    • Mantle Cell lymphoma

  o Chronic Lymphocytic Leukemia 10 months improvement in PFS
    • Best results achieved with RFC (rituximab fludarabine-cyclophosphamide)

• Better short term efficacy of radioconjugated anti CD20 mAb
  o Still 15% of CR with Zevalin in Follicular Lymphoma refractory to rituximab
Success and limitations of trastuzumab or cetuximab

• Trastuzumab
  o EGFR / HER-2 hyperexpressed by 20-30% of breast cancer
  o Initially developed as monotherapy of metastatic cancers
    • 30% of objective response in metastatic cancer, in first line; < 15% beyond
  o Used as adjuvant combined with taxane or anthracyclines
    • 4.8% of improvement in OS at 4 years

• Cetuximab
  o EGFR/ HER-2 expressed by 60-100% of epithelial cancers
  o Limited efficacy as monotherapy
    • < 10% of response in metastatic colo-rectal carcinoma
  o Used as 1st line treatment of advanced or metastatic squamous cell head & neck C.
  o Used as second line treatment of metastatic colo-rectal carcinoma
    • bevacizumab (anti VEGF-R) is usually preferred as first line treatment
    • Combination of cetuximab to bevacizumab does not improve/worsens PFS and increase toxicity
    • No improvement of PFS or OS as adjuvant treatment combined with chemotherapy
What do we know about rituximab mechanism of action?

• Apoptosis induced by CD20 binding
  o Shown in vitro with some but not all NHL cell lines

• Complement Dependent Cytotoxicity (CDC)
  o Lysis by MACs but also after binding to Complement Receptors by cell dependent lysis
    • Mediated by macrophages, neutrophils and NK cells

• Antibody Dependent Cell Cytotoxicity (ADCC)
  o Importance demonstrated in mice KO for FCγ receptor
  o Relevance demonstrated in patients by prognostic value of Fc γ RIIIa polymorphism
    • Better response to rituximab in FCGR3A-158V homozygous patients with FL
      ▪ Cartron G et al Blood 2002; 99: 754
    • Confirmed in other studies and with cetuximab and traztuzumab in other cancers
  o Mainly mediated by NK cells in human (as shown ex vivo on PBMC)
    • While macrophages play an important role in rodents
Mechanisms of rituximab-mediated cell death.

## Fcγ receptors

### Mouse

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<th>Activating Fc receptors</th>
<th>Inhibitory Fc receptor</th>
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### Human

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<td>FcγRIIB&lt;sup&gt;322T&lt;/sup&gt;</td>
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What do we know about rituximab pharmacology?

- Correlation between rituximab circulating levels and NHL response
  - Shown in low grade NHL by measuring serum levels during/after the treatment
    - After the 1st and before the 4th infusion
    - 1, 2 and 3 months after the 4 injections
  - Correlation between serum levels and PFS established by another group

- Debated impact of tumor burden on rituximab levels in patients
  - Current imaging techniques do not allow accurate assessment of NHL burden

- Maximal Tolerated Dose (MTD) of rituximab has never been identified
  - No rationale supporting the choice of the dose and treatment schedule
    - 375 mg/m²/week x 4
Numerous mechanisms of resistance to rituximab

- Related to the tumor
  - Down modulation of the target antigen
  - Shedding by metalloproteinase of the microenvironment
  - Shaving of the mAb-ligand complex from the surface of the tumor
  - Inhibition of apoptosis by hyper-expression of Bcl-2

- Related to the host
  - Defavorable polymorphism of FCRIIIa, CD11b and may be also of KIR

- Related to the antibody
  - Low trough levels
NEW TRENDS WITH mAb DEVELOPMENT in ONCOLOGY

1. IMPROVEMENT OF CYTOTOXIC mAbs
Improvement of cytotoxicity

To modify monoclonal antibody:
- Mutagenesis
- Modification of glycosylation

To increase CD20 expression:
- TNF-α, INF-α, GM-CSF, IL-4

To increase apoptosis:
- Chemotherapy (cisplatin, FAMP)
- Glucocorticoids

To increase complement activity:
- To increase affinity between Fc portion and C1q
- Bispecific MoAb (anti-CD59/CD20)

To increase cytotoxicity:
- Cytokines: IL-2, INF-α, IL-12, GM-CSF, G-CSF
- To increase affinity between Fc portion and FcγR
Improvement of cytotoxicity

- Modification of Fc fragment promoting the interaction with FcγR or C1q
  - Various goals
    - Improved binding to FcγRIIIA, FcRn or C1q
  - Various ways of engineering
    - A-fucosylation or other glycoengineering (e.g. development of anti CD20 GA-101)
    - Substitution of AA by mutagenesis
Improvement of CD20 mAb

History of anti-CD20 mAb in clinical translation

Lim SH et al Haematologica 2010; 95: 135-43
improvement of cytotoxicity

- Modification of Fc fragment promoting the interaction with FcγR or C1q
  - Various goals

- Enhancement of ADCC by stimulation of effector cells
  - Administration of IL-2 or GM-CSF
    - IL-2 expands and activates NK cells
    - GM-CSF induces differentiation of monocytes and activates neutrophils
      - Cartron G et al J Clin Oncol 2008; 26: 2725
  - Administration of thalidomide, lenalidomide or other IMiDs
  - More remote strategies with experimental mAbs or TLR agonists
Promotion of ADCC by modulation of effector cells

Houot R et al Trends Immunol 2011; 32: 510
NEW TRENDS WITH mAb DEVELOPMENT in ONCOLOGY

2. IMMUNOMODULATING mAbs
Why immunomodulating mAbs?

• Avoids constraints related to the target Tumor Associated Antigens (TAA)
  o Problems of density
  o Down modulation, shedding, shaving
  o Cross reactivity with normal host tissues

• Exploits the unique potential of immunosurveillance mechanisms
  o Innate and adaptive immunity
    o Mainly T cells (with ipilimumab) and NK cells (with anti KIR IPH 21 mAbs)

• Advantage of NK cells?
  • Limited risk of auto-immune reactivity
  • Frequency among total lymphocytes
  • Serial killers!
anti CTLA-4 mAb: mechanism of action

T cells and tumor cells

CTLA-4 and T cell activation

Fong L and Small EJ J Clin Oncol 2008; 26: 5275
ipilimumab phase III trial: improvement of Overall Survival

- 676 unresectable stage III or IV melanoma
- Ipi + vaccine (gp100 peptide) vs Ipi alone vs placebo

Ipilimumab: other results and development

- Metastatic melanoma
  - Combination with dacarbazine: 10% improvement of OS at 12 months in phase III
  
  - Combination with a B-RAF inhibitor (vemurafenib) successful in phase III?
  
  - Combination with other immunomodulating mAbs
    - e.g. anti PD1 or PDL1 mAbs
  
  - Combination with new cancer vaccines (more effective than gp 100 peptides)?

- Hormone Refractory Prostate Cancer
  - Ongoing phase III trials

- Many other solid tumors
  - Ongoing phase II trials
NK-cells and receptors

From Scientific Rationale to Drug Candidate

Blocking NK inhibitory receptor (KIR)

MHC from transplanted patient is not recognized by KIR of NK cells from donor due to genetic difference between donor and recipient

=> NK cell is activated

Mimicking mismatch situation with a blocking antibody targeting KIR

=> NK cell is activated

NK cell from donor

NK and tumor cells from patient

IPH 2101 the anti-KIR antibody
Human inhibitory receptors: Different KIR exist (expression is variegated) and ligands are polymorphic (Moretta et al., 1996, Annu Rev Immunol, 14:619)

Human NK inhibitory receptors

- HLA-Bw4
- HLA-A
- HLA-Cw4 like C2 type
- HLA-Cw3 like C1 type

Mouse NK inhibitory receptors

- H2Kb, Kd
- H2Dd

Lectin like domain
Immunoglobulin domain
NK cell cytotoxicity and cancer risk

Imai K et al Lancet 2000; 356: 1795-9
KIR ligand mismatch: impact on relapse risk and DFS

Ruggeri L et al Blood 2007
Drug candidate characterization

- High affinity for KIR2DL1/2/3
- Blocking to C1 and C2 HLA alleles (all MHC class I C alleles)
  
  \[\rightarrow\text{ Product can be used in all individuals irrespective of MHC haplotype} \]

  \[
  \text{Targets a major population of NK cells (mean 40% of all NK population)}
  \]

- IgG4 isotype (blocking mAb)
- Proprietary epitope (additional IP)
Pharmacology of anti-KIR IPH2101 (1-7F9) drug candidate

Effect on NK-mediated lysis of AML blasts in vitro

NK from HLA-matched healthy donor

<table>
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<tr>
<th>E:T Ratio</th>
<th>- mAb</th>
<th>+ 1-7F9</th>
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<td>50</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
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</tbody>
</table>

NK from patient in remission (autologous situation)

Romagne et al, 2009, Blood, 24;114(13):2667-77
STRATEGY OF DEVELOPMENT OF NEW mAbs
Pre-clinical studies

- **In vitro / ex vivo** studies with human cells and tissues
  - Functional assays with PBMC and tumor cells
    - e.g. ADCC
  - Tissue distribution of the target

- **In vivo** studies in relevant animal species
  - Pharmacology and toxicology
    - Dose toxicity relationship
    - No Adverse Effect Level (NOAEL)

- Main issue: the species barrier
  - Specificity of the target
    - Anti human CD20 mAbs do not recognize rodent CD20; KIRs do not exist in rodent
  - Difference of sensitivity between human and non human primates
    - Monkeys release less easily pro-inflammatory cytokines
  - Difference of pharmacokinetics between species
Pharmacokinetics

• Administration by Intra Venous or Sub Cutaneous route

• Poor diffusion in profound tissues
  - Levels often 10 times lower as compared to serum
    - A few exceptions: tissues with fenestrated capillaries such as bone marrow

• t ½ of IgG1, IgG2 and IgG4: around 3 weeks

• Wide variations of the clearance
  - Dose-dependent clearance
    - Protection of proteolysis by FcRn expressed by endothelial cells and APC

  - Target-mediated drug disposition
    - Explains the impact of tumor burden on rituximab serum levels
      - Daydé D et al Blood 2009; 113: 3765

  - Immunization against mAb
Rituximab and tumor burden

- Importance of tumor burden shown in transgenic mice expressing CD20

Daydé D et al Blood 2009; 113: 3765
Safety: on target effect

• Related to a transient agonist effect
  o Immune activation and cytokine release syndrome (rituximab, alemtuzumab)

• Related to the mAb mechanism of action
  o Blockade
    • CTLA-4 autoimmunity (ipilimumab)
    • VEGF HTA or hemorrhages (bevacizumab)
  o Depletion
    • CD20/CD52 opportunistic infections (rituximab, alemtuzumab)

• Related to target expression in healthy tissues
  o EGF-R/HER-1 skin reactions of various severity (cetuximab …)
  o EGF-R/HER-2 cardiotoxicity in > 5% of the patients (trastuzumab)

• Related to target expression in tumor tissue
  o The tumor lysis syndrome (leukemia/lymphoma) (rituximab/alemtuzumab)
The TEGENERO story: the medical history

- 6 healthy volunteers treated concomitantly at the first dose level

**Figure 1. Summary Timeline of the Main Events after Infusion of TGN1412.**
The course is divided into four phases: cytokine storm, reactive, recovery, and steady state. ALT denotes alanine aminotransferase. Dashed lines represent the responses of Patients 5 and 6 (who were the most seriously ill).

The TEGENERO story: the cytokine storm

Lessons of TEGENERO story

• Importance of *in vivo/ ex vivo* studies conducted with human cells
  o Enable comparison of sensitivity with toxicology species

• More stringent rules for the choice of the first dose in human
  o Identification of factors of risk for safety
    • Mechanism of action of the drug
      ▪ Targets with pleiotropic effects
      ▪ Cytokine release or other biological cascade
    • Tissue distribution of the target
    • Lack of relevant animal species

  o Use of the MABEL (Minimal Anticipated Biological Effect) rather than the NOAEL

*EMA guidelines from September 1st 2007*

*Guidelines on strategies to identify and mitigate risks for first in human clinical trials with investigational medicinal products*
mAbs (ipilimumab) and autoimmunity

• Dose dependent efficacy and toxicity
  o Up to 25% of grade 3-4 AE at the highest tested dose
• 10-15% of immune related grade 3-4 AE in the phase 3 registration trial
  o With ipilimumab + vaccine vs 3% with the vaccine alone (metastatic melanoma)

• Very broad autoimmune toxicity
  o Skin disease (rash, pruritus, vitiligo)
  o Colitis
  o Hepatitis
  o Peripheral neuropathy
  o Endocrine disorders (mainly related to hypophysitis)

• Significant but non absolute correlation between autoimmunity and response
  • Attia P et al J Clin Oncol 2005; 23: 6043
mAbs (anti CD20 and CD52 Abs) and infections

- Increased risk documented with mAbs depleting lymphocytes

- Anti CD20 mAbs (rituximab and others)
  - Profound and long lasting depletion of B lymphocytes
    - Hypoglobulinemia is however unusual
  - Limited infectious over risk
    - Rare reactivation of HBV (fulminant hepatitis) and JC virus (multifocal leukoencephalopathy)

- Anti CD52 mAbs (alemtuzumab)
  - Depletion of both T and B lymphocytes
  - Frequent opportunistic infections related to T cell depletion (and low CD4 cell count)
    - Frequent reactivation of herpetoviridae
  - Frequent bacterial sepsis
Safety: off-target toxicity

• Immunogenicity of mAb
  o More important with mouse derived mAb
    • However a presisting risk with fully human mAb
      ▪ mAb triggers an homeostatic anti idiotype immunization

  o Triple theoretical risk
    • Allergy and anaphylaxis (Gell & Coombs type I)
    • Serum sickness (Gell & Coombs type III)
      ▪ Very rare with mAbs used in oncology
    • Accelerated clearance of mAb
      ▪ Loss of effect with repeated administration

• Activation Fc-dependent of immune effectors
  o Uncertain relevance with mAbs
**Efficacy: several limitations to take into account**

- **Tumor burden**
  - Lower mAb concentrations because of the target mediated drug disposition
  - Unfavorable effector: target ratio
    - e.g. resistance to alemtuzumab of bulky CLL
  - Exhaustion / dysfunction of NK cells
    - in AML at the diagnosis
      - CD16 expression and ADCC are however preserved
      - Dysfunction is reversible with disease remission
        - Documented as early as D30 after induction in patients in CR

- **Combined drugs**
  - Could expand NK cells and enhance ADCC
    - e.g. thalidomide and lenalidomide; the latter is developed with rituximab in NHL
  - Could impair NK or other effector cell survival or function
    - e.g. cytotoxic drugs depleting NK cells
An example of “surge” of NK cells after a lympho-depletive chemo

From Boyadizis M et al  Biol Blood Marrow Transplant 2008
Efficacy: usual clinical end points

• Short term end points
  o Objective response is usually required
    - Solid tumors
      ▪ > 30% of decrease in the larger diameter of the lesions
    - Hematological malignancies
      ▪ Specific to each disease
  o Complete response can be essential
    - In acute leukemia, partial response has no impact on survival

• Long term end points
  o Overall survival
    - OS
    - Usually required for registration
  o Progression Free survival
    - PFS
  o Disease Free survival
    - DFS
clinical end points: current challenges

• Relationship between Response and Survival
  o Depends on disease kinetics
    • Interest of CR is still debated in slowly progressing disease
      ▪ e.g. in rituximab trials conducted with low grade NHL
        • Salles GA Hematology Am Soc Hematol Educ Program 2016: 216
  o Depends on mAb mechanism of action
    • Impact of ipilimumab on response in melanoma is limited
      ▪ Responses are infrequent (10-15%), usually partial and often delayed
        • Wolchok JD et al Cin Cancer Res 2009 15: 7412

• Eradication of Minimal Residual Disease
  o Would better correlates with cure than Complete Response
  o Remains however very difficult to document
    • Molecular quantification by RQ PCR of genetic abnormalities associated to cancer cells
      ▪ e.g translocation of BCR ABL in Chronic Myeloid Leukemia
Ipilimumab: residual issues

• Identification of the optimal dose
  o 10 mg/kg seemed to be more effective than 3 mg/kg

• Better understanding of the relationship between response and survival

• Identification of biomarkers predictive of the response

• Interest of a maintenance therapy by quarterly injections
  o Tested in phase III trials without conclusive results

• Interest of combinations with other immunomodulating Abs
  o Efficacy … and safety
Phase I clinical trials

- Limited sample size (n = 10 to 30)
  - Dose escalation design remains widely used
    - Doubling of the cohort in case of Dose Limiting Toxicity (DLT)
    - Assessment is often but not always limited to single doses

- Primary objective
  - Safety:
    - Identification of DLT, if any
    - By grading the severity of adverse event with a NCI scale
  - Pharmacology
    - PK and occupancy of the mAb molecular target

- Issues
  - Not only the choice of the lowest dose
  - But also the choice of the maximal dose
    - Maximal Tolerated Dose (MTD) is not always sought with immunomodulating drugs
  - And the administration schedule in trials with repeated doses
Phase II clinical trials

- Larger sample size (N=20 to >100)
  - Design is often but not always randomized
    - Between one or several doses of the experimental mAb and/or a placebo
- Primary objective
  - Documentation of the clinical activity (« efficacy »)
    - The goal being to reject mAb which would be sufficiently active
      - CR rate < to the results achieved with the reference treatment
    - However, sample size is excessively limited to allo comparison between different arms
  - Safety assessment remains an important secondary objective
- Issues
  - mAbs are often developed as combination with « standard of care » treatment
    - The efficacy of the standard of care limit the interpretation of the results
  - Immunomodulating mAbs are often developed as maintenance in previously treated patients
    - Relatively long term end points (such as DFS) should be used in disease already in CR
CONCLUSIONS
The last 13 years (since 1997)

• mAb became one of the most active field of development in onco-pharmacology
  o More than 100 mAbs in development

• Naked cytotoxic mAb are relatively easy to develop … however:
  o mAbs are no longer regarded as « innocent » molecules
    • Lessons of Tegenero story
  o Results achieved until now remain relatively modest
    • Improvement of 10% or less of the survival with rituximab and trastuzumab

  o Immuno-pharmacology should be better assessed to improve their efficacy
    • Pharmacokinetics ?
    • Interaction with immune effector cells especially NK cells
      ▪ Engineering of Fc fragment enhancing their binding to Fc receptors
      ▪ Enhancement of effector cells by small molecules, cytokines … or mAbs
And now?

- Two ways of development have just emerged ... to achieve superior results?

① New Antibody Drug Conjugates (ADC)
   - Recent registration of brentuximab (Acetris®) a new anti CD30 ADC in lymphoma
   - Might be especially useful as « debulking » drugs

② Immunomodulating mAbs
   - Recent registration of ipilimumab (Yervoy®) anti CTLA4 mAb in metastatic melanoma
     - Ongoing development of other mAbs enhancing T cell activity
       - Limiting auto-immune mediated reactions
   - Unique interest of mAbs enhancing NK cell activity?
     - Example of IPH21 anti KIR mAb
       - Might be used to enhance cytotoxicity of NK cells alone or ADCC mediated by NK cells
   - Specific constraints linked to the development of these mAbs ... including the status of NK cells...
   - Potential interest of combinations between several immunomodulating mAbs