INSTITUT PASTEUR

UNIVERSITE PIERRE & MARIE CURIE PARIS 6

The 2nd PSU International Teaching Platform on Tumour Immunology and Immunotherapy

Jointly organized by
Prince of Songkla University, Université Pierre et Marie Curie (Paris 6) and Institut Pasteur

December 15 – 20, 2003
At The Department of Biomedical Sciences
Faculty of Medicine, Prince of Songkla University,
Hat Yai, Songkhla, Thailand

Lecture 6:
Specific therapy – monoclonal antibodies
Prof. Catherine Fridman

December 16, 2003

FUNCTIONS OF ANTIBODIES

IgM PRESENT IN BODY FLUIDS AND TISSUES,
DEFENSES AGAINST INFECTION AND CANCER

IgG PRESENT IN BODY FLUIDS AND TISSUES,
DEFENSES AGAINST INFECTION AND CANCER

IgA PRESENT IN MUCOSAL SURFACES,
NEUTRALIZATION OF PATHOGENS

IgE PRESENT IN TISSUES AND ON VASCULAR ENDOTHELIUM,
ALLERGY, DEFENSES AGAINST HELMINTHS

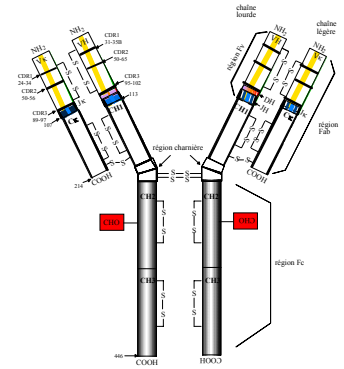
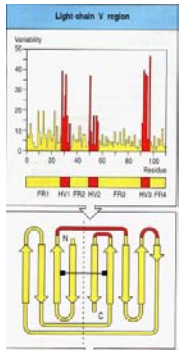
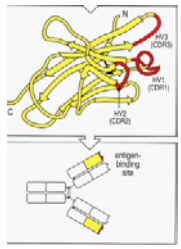


Fig. 1. Schéma d'une IgG de souris.
-S-S-, ponts disulfine; les chiffres indiquent les positions des acides aminés C, région constante; CHO, carbohydrate; COOH, extrémité carboxy-terminale H, chaîne lourde; L, chaîne légère; NH2, extrémité amino-terminale; V, région variable.





ISOTYPES HAVE DISTINCT BIOLOGICAL ROLES

	IgM	IgG	IgA	IgE
COMPLEMENT ACTIVATION	+	+	-	-
NEUTRALISATION OF PATHOGENS	-	+	+	-
OPSONISATION	-	+	±	-
ADCC	-	+	-	-
ACTIVATION OF MAST CELLS	-	-	-	+

POLYCLONAL ANTISERUM

B1 → Ab1
B2 → Ab2
B3 → Ab3

MONOCLONAL ANTIBODY

B1 → Ab1

MYELOMA CELL LINES

MOUSE MYELOMA MOPC21

P3X63 Ag8, Sp 2/0 } NON Ig SECRETING, HGPRT NEGATIVE

RAT MYELOMA

EBV-TRANSFORMED HUMAN LYMPHOMA LINES

	MOUSE ORIGIN	HUMAN ORIGIN
CHIMERIC MAbs	VARIABLE REGIONS	CONSTANT REGIONS
HUMANIZED MAbs	HYPERVARIABLE REGIONS	FRAME WORK AND CONSTANT REGIONS
HUMAN MAbs IN XENOMOUSE	NONE	WHOLE MAb

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THERAPEUTIC MAbs

AUTOIMMUNITY (PAR)	ANTI TNFα ANTI IL8	TNFα IL8
TRANSPLANTATION	OKT3	CD3 (T cells)
ALLERGY	ANTI-IgE ANTI-FcεRI	IgE FcεRI
GLAUCOMA	ANTI-TGFβ	TGFβ

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Oncology antibodies approved by US FDA

Table 1

Oncology antibodies approved by the US FDA.

Name (US tradename*)	Company	Target	Mechanism	Antibody form†	Cancer indication	US FDA approval date	Refs
Rituximab (Rituxan®)	Genentech and IDEC Pharmaceuticals	CD20	ADCC, CDC, directly induces apoptosis	Chimeric IgG1	NHL	11/26/97	[4]
Trastuzumab (Herceptin®)	Genentech	HER2	Inhibition of HER2-mediated tumor cell proliferation and migration	Humanized IgG1	Breast cancer	9/25/98	[5]
Gemtuzumab ozogamicin (Mylotarg®)	Wyeth-Ayerst and Celltech Group	CD33	Delivery of calicheamicin into leukemic cells resulting in DNA strand breaks and apoptosis	Humanized IgG4 linked to calicheamicin	AML	5/17/00	[6]
Alemtuzumab (Campath®)	Ilex Pharmaceuticals and Berlex Laboratories	CD52	ADCC, CDC	Humanized IgG1	CLL	5/7/01	[7]
Ibritumomab tiuxetan (Zevalin™)	IDEC Pharmaceuticals	CD20	Delivery of cytotoxic radiation, ADCC, CDC, apoptosis	Murine IgG1 ^{90}Y conjugate (murine parent form of rituximab)[2]	NHL	2/19/02	[8]

*The suffixes of the generic name of antibodies are assigned as follows: murine antibodies are 'omab', chimeric antibodies are 'ximab', humanized antibodies are 'zumab', and fully human antibodies are 'umab'. †IgG1 and IgG2 isotypes are effective in inducing CDC and ADCC, whereas the IgG4 isotype is not effective for either. IgG2 has CDC activity, but no ADCC activity [9]. [2]Rituximab was administered preceding Indium-111 Zevalin followed seven to nine days later by a second infusion of Rituximab prior to Yttrium-90 Zevalin. AML, acute myelocytic leukemia; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin's lymphoma.

(Trikha, Cur Opi Biotechnology, 2002)

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Oncology antibodies in phase III clinical development (1)

Table 2

Oncology antibodies in phase III clinical development.

Name	Company	Target	Mechanism	Antibody form	Cancer indication	Status	Refs
Cetuximab (Erbitux™)	ImClone Systems and Bristol Myers Squibb	EGFR (HER1)	Inhibits EGFR-mediated tumor cell invasion, proliferation, metastasis, and angiogenesis Enhances activity of some chemotherapeutics and radiotherapy	Chimeric IgG1	Colorectal cancer	BLA not accepted for review by the FDA	[28]
Tositumomab/ ^{131}I-tositumomab (Bexxar®)	Coriaia and GlaxoSmithKline	CD20	Delivery of cytotoxic radiation, ADCC, CDC, apoptosis	Murine IgG2a ^{131}I conjugate plus unlabeled antibody	NHL	BLA not approved by the FDA, under appeal	[8]
Bevacizumab (Avastin™)	Genentech	VEGF	Inhibition of VEGF-induced angiogenesis	Humanized IgG1	Breast cancer, colorectal cancer, NSCLC, renal cancer	Phase III	[29]
Cotara™ TNT-1 ^{131}I-chTNT-1/B	Peregrine Pharmaceuticals	DNA-associated antigens in the nucleus	Targets dead and dying cells The drug binds to DNA or DNA-associated proteins and concentrates toxic radioactivity within the tumor	Chimeric Ig ^{131}I conjugate	Glioma	Phase III	

(Trikha, Cur Opi Biotechnology, 2002)

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Oncology antibodies in phase III clinical development (2)

BEC2 (Mitomomab)	ImClone and Merck KGaA	GD3	Anti-idiotypic vaccine to target ganglioside GD3, administered with BCG as an immune stimulant	Murine IgG2a	SCLC	Phase III	
Zamyl™ (SMART™ M195)	Protein Design Labs	CD33	ADCC, CDC	Humanized IgG1	AML	Phase III	
Pemtumomab (Theragyn)	Antisoma and Abbott	PEM (MUC1)	Delivery of toxic radioactivity to PEM-expressing tumor cells	Murine IgG1 ^{90}Y conjugate	Ovarian cancer	Phase III	
CeaVac™	Titan Pharmaceuticals	CEA	Anti-idiotypic vaccine antibody to target CEA	Murine IgG	Colorectal cancer	Phase III	
OvaRex®	Altarex	CA 125 antigen	Induces immune response against tumor-expressed CA 125	Murine Ig	Ovarian cancer	Phase III	
LymphoCide™ (Epratuzumab)	Immunomedics and Amgen	CD22	Binds and clears CD22 expressing cells	Humanized Ig	NHL	Phase III	[8]

This information in these tables was compiled from a variety of sources including publications, scientific meeting presentations, and company websites. All efforts were made to make the tables complete and accurate, but there is no guarantee. Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; AML, acute myelocytic leukemia; BCG, Bacillus Calmette-Guerin; BLA, Biologics Licence Application; CDC, complement-dependent cytotoxicity; CEA, carcinoembryonic antigen; EGFR, epidermal growth factor receptor; NHL, non-Hodgkin's lymphoma; NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer; VEGF, vascular endothelial growth factor.

(Trikha, Cur Opi Biotechnology, 2002)

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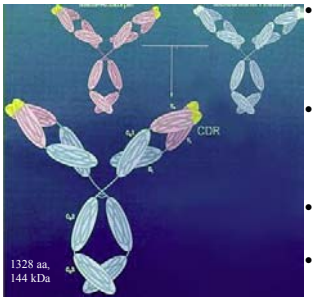
HERCEPTIN: anti-HER2neu

- **Humanized mAb**
- **HER2neu, an oncogene over-expressed in breast cancer, ovarian cancer**
- **Encodes a receptor for a growth factor (EGF-R family)**
- **Induces internalization of the receptor, thus decreases sensitivity to growth factor**

Rituximab: Anti-CD20

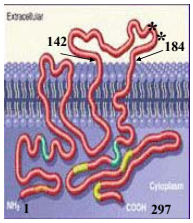
Mouse antibody (2B8) Human antibody (IgG1κ)



Chimeric antibody (IDEC-C2B8)

- Chimeric antibody:
  - mouse variable regions
  - human constant regions (IgG1, kappa)
- Retains capacities
  - To bind to human FcγR
  - To activate the human complement cascade
- No induction of human immune response
- Long plasmatic half life (205 h after the 4th perfusion)

CD20: Structure

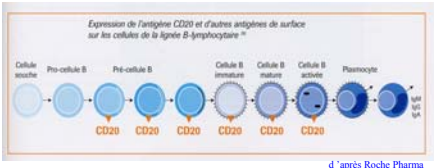


Tedder at al, Immunol Today 1994
Riley et al, Semin Oncol 2000
Deans et al, Immunology 2002

- Chromosome 11
- 297 amino acids, 33 to 37 kDa
- 4 spanning domains
- associated with lipid rafts (aa 215-229)
- No known ligand
- No precise function but involved
  - in B-cell activation
  - in the regulation of B-cell growth and of
  - transmembrane calcium flux

11q12-q13.1

CD20: Expression

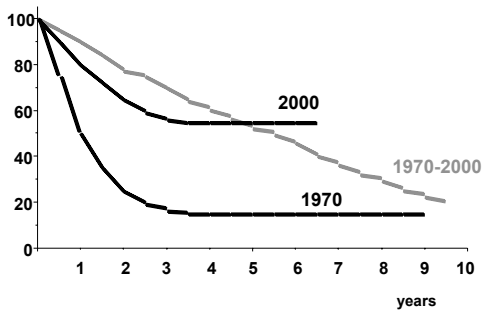


d 'après Roche Pharma

- Expressed from pre-B cell to mature and activated B cells
- Expressed on nearly 95% of B-cell NHL
- No circulating soluble form in plasma
- Not down-modulated, shed or internalized following binding of antibody

Survival of non-Hodgkin's advanced stage lymphomas

LNH low malignancy *vs* **LNH high malignancy**



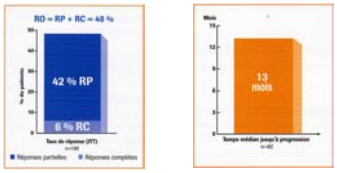
rituximab (anti-CD20)
in low grade B cell lymphomas

Rituximab in low grade B cell lymphoma

		No. pts (eval.)	response rate	
relapse/non-responders				
Maloney (1997)	follic./lymphocyt.	34	50%	3 RC, 14 RP
McLaughlin (1998)	follic./lymphocyt.	151	50%	9 RC, 67 RP
Piro (1999)	follic./lymphocyt.	35	60%	5 RC, 16 RP
Davis (1999)	follic./lymphocyt.	28	43%	1 RC, 11 RP
Nguyen (1999)	lymphocyt.	15	7%	0 RC, 1 RP
1st line of treatment *(low tumoral mass)*				
Colombat (Blood 2001)	follic.	49	73%	13 RC, 23 RP
rituximab + CHOP x 6 *(1st line and relapses)*				
Czuczman (1999)	follic./lymphocyt.	40	95%	22 RC, 16 RP

Efficacy of Rituximab therapy in B-cell lymphoma (I)

- Treatment of relapsed or refractory patients with low-grade or follicular lymphoma



(Mc Laughlin, J. Clin Oncol 1998)

Rituximab (anti-CD20) in aggressive B cell lymphomas

Efficacy of Rituximab therapy in B-cell lymphoma (II)

- Treatment of diffuse large B-cell lymphomas with a combination of CHOP plus Rituximab in elderly patients

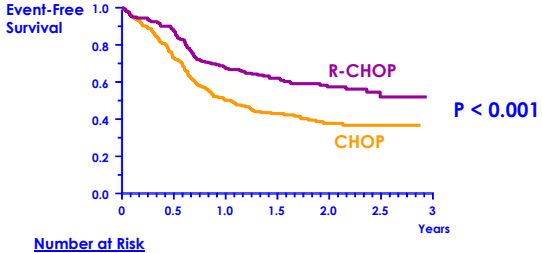
CHOP compared with CHOP plus Rituximab- LNH-98.5

Cyclophosphamide 750 mg/m²
Doxorubicine 50 mg/m²
Vincristine 1.4 mg/m²
Prednisone 40 mg/m²/d x 5 d

3 weeks — **8 cycles**

CHOP
Rituximab 375 mg/m²

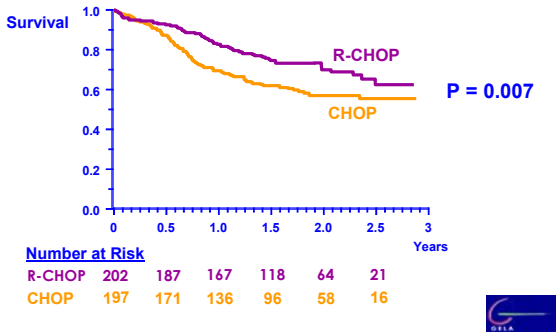
Event-free survival



Number at Risk

R-CHOP	202	177	137	108	63	19
CHOP	197	144	99	71	41	17

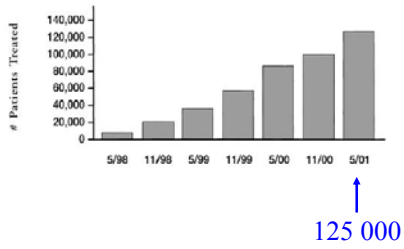
Overall survival



Number at Risk

R-CHOP	202	187	167	118	64	21
CHOP	197	171	136	96	58	16

Increasing number of patients treated with Rituximab between 1998 and 2001 in USA



125 000

In vitro mechanisms of action of Rituximab™ on primary non-Hodgkin's lymphomas ?

The anti-CD20 therapy leads to *in vivo* depletion of B cells

- Reff et al, Blood 1994 (Macaque):
  - in Peripheral blood (98%, D8),
  - in Lymph Nodes and Bone Marrow (95%, D36).
- Maloney et al, Blood 1994 (Human):
  - in Peripheral blood B cells (90%, D3-M2),
  - in Lymph Nodes: 80% of positive tumour cells with reduction of cell number (D14).

THERAPEUTIC ANTITUMOR MAbs MECHANISMS OF ACTION

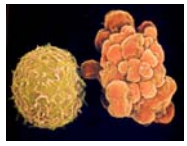
1 -APOPTOSIS

2 -COMPLEMENT-DEPENDENT CYTOTOXICITY

3-ADCC via NK CELLS

4 - PHAGOCYTOSIS OF TUMOR CELLS VIA MACROPHAGES

Detection of apoptosis



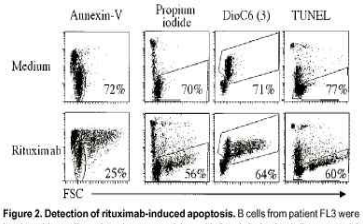
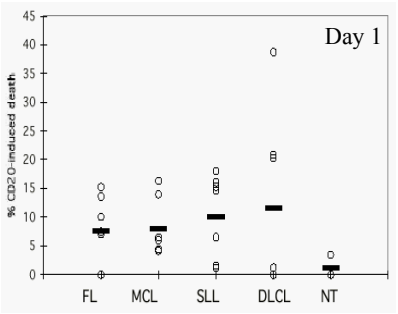
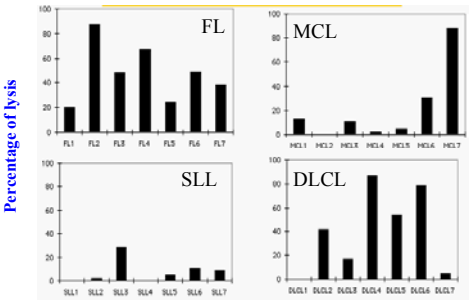


Figure 2. Detection of rituximab-induced apoptosis. B cells from patient FL3 were incubated in medium alone (upper panels) or with 2 μg/mL rituximab (lower panels) for 2 days. Cell death was analyzed using annexin V, PI, $DiOC_6(3)$, or the TUNEL assay. Percentages of gated cells are indicated.

The induction of apoptosis is weak

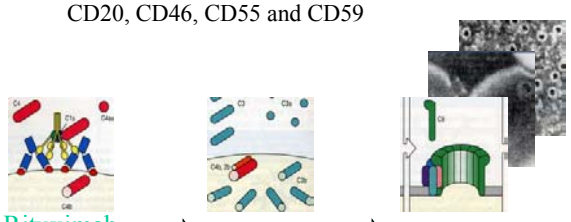


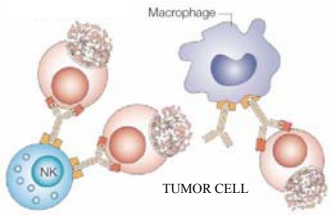
CDC is heterogeneous among the different groups of NHL



Non tumour cells are resistant to CDC

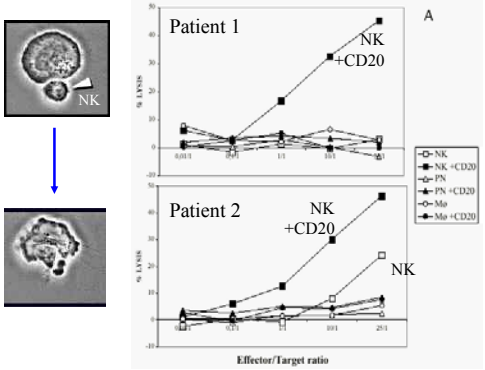
Regulation of CDC by CD20, CD46, CD55 and CD59





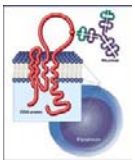
(from L. Chatenoud, Nature Rev. Immunol., 3, 123-132, 2003)

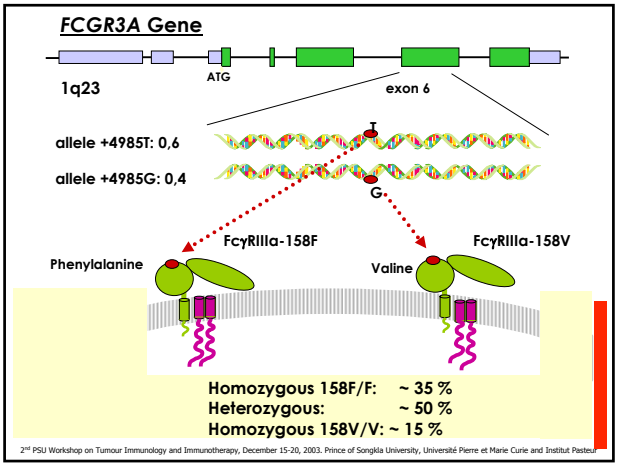
The ADCC is CD20-dependent



Rituximab therapy: What are the biologic criteria of efficacy prediction?

- expression of CD20
- ???





FCGR3A polymorphism influences binding of IgG to NK-FcγRIIIa

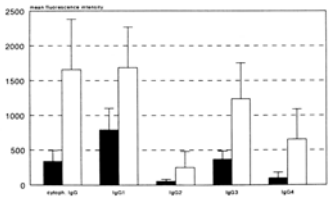
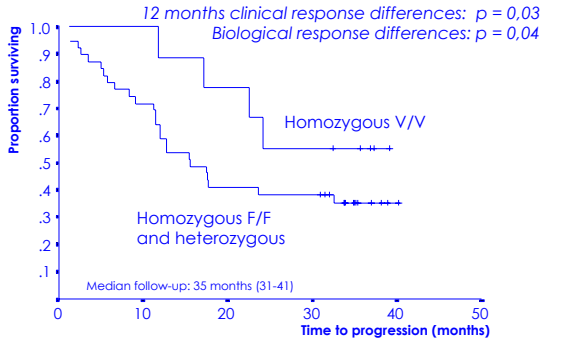


Fig 2. FcγRIIIa^{NK}-158V binds more IgG than does FcγRIIIa^{NK}-158F. IgG binding by NK cells from individuals either homozygous *FcγRIIIA-158FF* (■) or homozygous *FcγRIIIA-158VV* (□) was compared, irrespective of the FcγRIIIa-48L/R/H genotype. At least three different donors of each genotype were tested. The level of cytophilic IgG and the binding of IgG1, IgG3, and IgG4 was significantly higher in NK cell from *FcγRIIIA-158VV* individuals ($P < .05$ in all cases).

(independently from FcγRIIIa-48L/R/H genotype)

Koene HR et al. *Blood*, 1997.

Cartron et al., Blood 2002



Weng et Levy, JCO 2003

- Reanalysis of follicular NHL (n = 87) patients treated between 1993 and 2003.

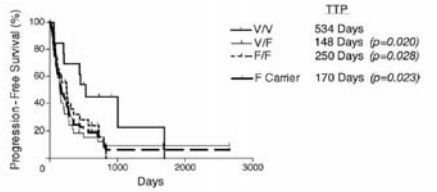
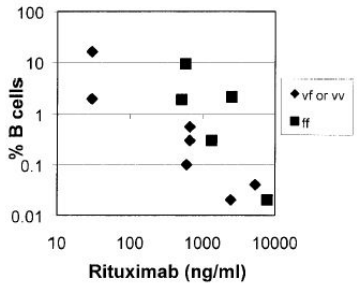


Fig 2. Kaplan-Meier estimates of progression-free survival by immunoglobulin G fragment C receptor IIIa (FcγRIIIa) 158 valine (V)/phenylalanine (F) polymorphism. Progression-free survival curves were plotted by FcγRIIIa 158 V/F genotype on all 87 patients. F carriers represent patients with either 158 V/F or 158 F/F genotype. TTP, median time to progression.

FCGR3A influences the decrease in B lymphocytes induced by Rituximab



Anolik JH et al. *Arthritis Rheum*, 2003.

Weng et Levy, JCO 2003

- **Influences of FcγRIIIa-158V/F polymorphism and FcγRIIa-131H/R polymorphism.**

Table 7. Prognostic Factors for Clinical Response: Logistic Regression Analysis

	1-3 Months			6 Months			9 Months			12 Months		
Characteristic	OR*	95% CI	P†	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
158 V/V	12.25	1.35 to 111.16	.026	8.48	1.54 to 46.60	.014	7.94	1.59 to 39.76	.012	17.14	2.94 to 100.18	.002
131 H/H	2.96	0.85 to 10.35	.090	8.03	2.13 to 30.21	.002	6.26	1.86 to 21.06	.003	7.25	1.89 to 27.84	.004
Stage III versus IV	0.66	0.19 to 2.22	.498	1.01	0.28 to 3.62	.984	0.62	0.16 to 2.40	.486	0.78	0.16 to 3.85	.759
Age > 60 years	3.08	0.57 to 16.76	.193	2.62	0.57 to 12.12	.217	1.73	0.35 to 8.53	.500	4.22	0.73 to 24.25	.107
Prior transplant therapy	0.86	0.30 to 2.43	.772	0.55	0.17 to 1.73	.304	1.24	0.39 to 3.95	.717	2.18	0.56 to 8.45	.261
Bulky disease	1.81	0.54 to 6.05	.336	0.62	0.17 to 2.27	.470	0.68	0.18 to 2.53	.563	0.47	0.11 to 2.14	.333
> 2 extranodal sites of disease	1.22	0.30 to 4.89	.784	0.32	0.06 to 1.69	.181	0.57	0.11 to 2.84	.489	0.23	0.03 to 1.87	.170

Abbreviations: OR, odds ratio; V, valine allele; H, histidine allele.
*Relative odds of response to rituximab treatment.
†Two-sided; considered statistically significant for $P < .05$.

HOW TO INCREASE ANTI-CD20 EFFICACY ?

INCREASE ANTIBODY-DEPENDENT CELL CYTOTOXICITY (ADCC)

- GM-CSF (Khouri et al.,2002; Rossi et al. 2002)
- Interleukin 2 (Holmberg et al.,2003)
- G-CSF (Van der Kolk et al.,2003)

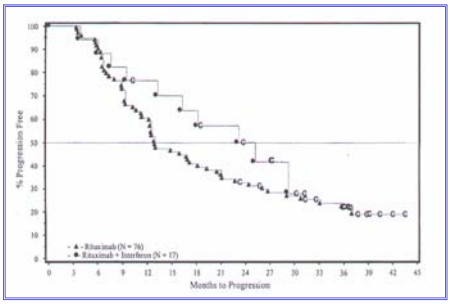
G-CSF + RITUXIMAB

- Rationale:
  - increase in ADCC by PMN
  - increase in CD11c and FcγRI expression
  - increase in PMN number
- Treatment:
  - G-CSF 5µg/kg X 3 days
  - Rituximab 375 mg/m^2 on day 3

  X4
- Results:
  - 19 pts with low grade NHL
  - ORR: 42%
  - Median TTP: 24 months (52+- 56+)

Van der Kolk et al.,Leukemia,2003

TIME TO PROGRESSION OF PATIENTS TREATED WITH IFNα 2a + RITUXIMAB



Comparison with the TTP of patients treated with Rituximab alone (from the pivotal study)

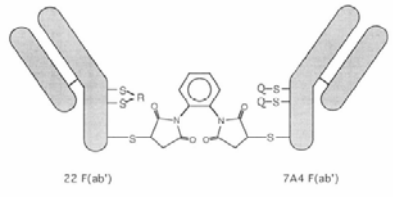
CONCLUSIONS

- A better knowledge of the mechanisms of action of MoAbs will allow to improve their efficacy
- Engineering CD20mAb :
  - At the antigen-binding site
  - At the Fc portion
- The future of immunotherapy is in combinations rather than in sequential treatments

BISPECIFIC ANTIBODIES

ACTIVATES AN EFFECTOR CELL

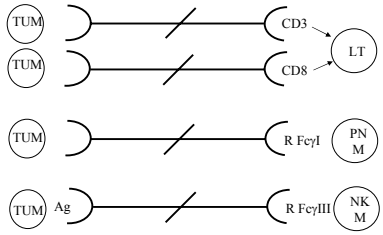
REACTS WITH THE TUMOUR CELL



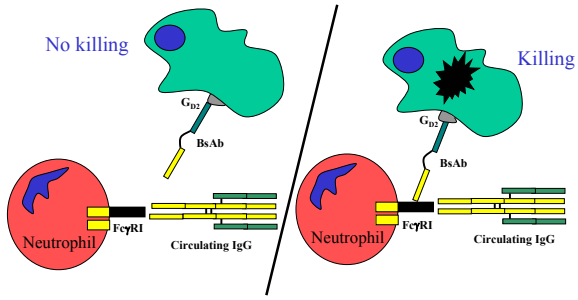
Schematic diagram of a bispecific Ab (MDX-260, anti-CD64/anti-G_{D2}).
Q represents N-ethyl succinimidyl; R, O-phenylenedissuccinimidyl.

(Michon et al., Blood, 86, 1124-1130, 1995)

BISPECIFIC MAbs



Targeting neuroblastoma cells with anti-FcγRI/anti-G_{D2} BsAb



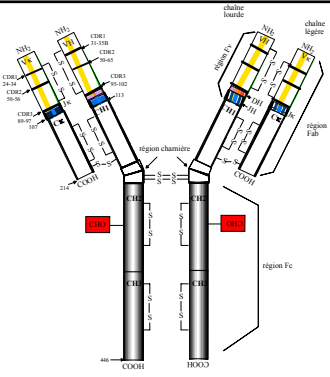
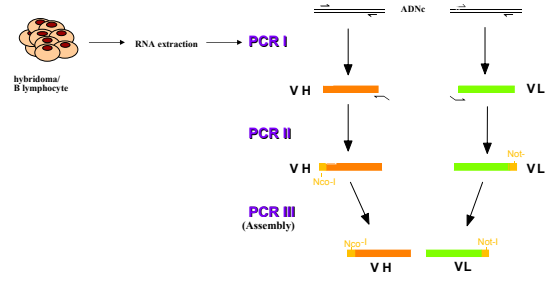


Fig. 1. Schéma d'une IgG de souris.
-S-S-, ponts disulfine; les chifres indiquent les positions des acides aminés C, région constante; CHO, carbohydrate; COOH, extrémité carboxy-terminale H, chaîne lourde; L, chaîne légère; NH2, extrémité amino-terminale; V, région variable.



Construction of a scFv

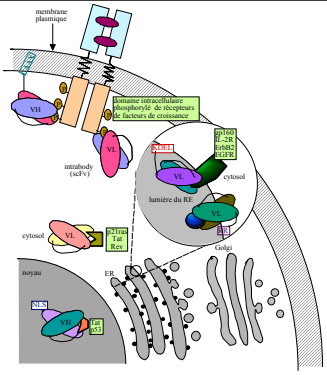
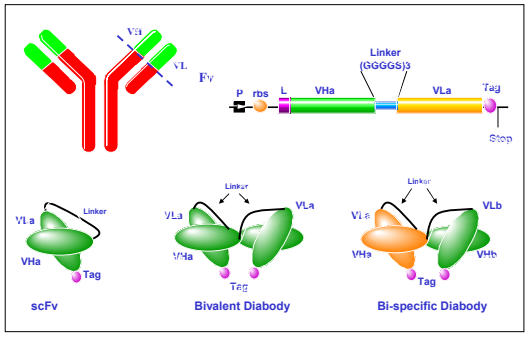


Fig. 7. Sites d'action potentiels des "intrabodies". Des scFvs localisés dans le cytosol sont utilisés pour neutraliser ou séquestrer des molécules de ce compartiment ou pour interagir avec les domaines intracellulaires de protéines transmembranaires. Le ciblage de molécules insérées dans la membrane plasmique peut être amélioré par addition de la séquence "CAAX". Les scFvs peuvent être dirigés vers le noyau grâce à une séquence "NLS" (nuclear localization signal), ou être envoyés dans la lumière du réticulum endoplasmique ("RE") grâce à l'utilisation d'une séquence signal et de peptides KDEL ou RR.



	ADVANTAGES	PITFALLS
MAb	LONG HALF LIFE	SIZE
	HIGH AFFINITY	NEED Ag FOR IMMUNIZATION
		HYBRIDOMA INSTABILITY
		IMMUNOGENICITY
ScFv	DO NOT NEED IMMUNIZATION	LOW AFFINITY
	CAN BE EXPRESSED	MONOVALENT
	INTRACELLULARLY	SHORT HALF LIFE