SOCIEDAD ESPAÑOLA DE HEMATOLOGÍA Y HEMOTERAPIA

FEHH

Tratamiento antineoplásico en Hematología

"Mucho que aprender, mucho que recordar"

Anticuerpos monoclonales

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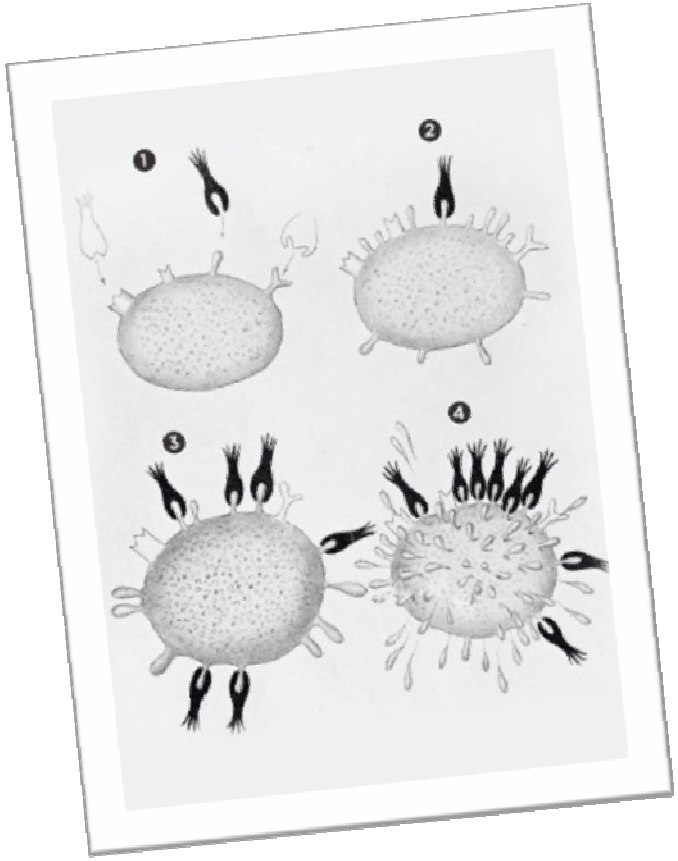
Breakthrough of the Year 2013

Historia de la inmunoterapia

1891 After reading that a patient's tumour disappeared after a bacterial infection, surgeon William Coley begins injecting cancer patients with bacteria (now known to be *Streptococcus pyogenes*).

1909 Biologist Paul Ehrlich suggests that immune cells actively patrol the body to suppress the growth of cancerous cells.

Nace el concepto de la inmunoterapia



Las "balas mágicas" de Ehrlich

Historia de la inmunoterapia

Niels K Jerne

Georges JF Köhler

César Milstein

1984 – Nobel Prize in Physiology and Medicine

"for theories concerning the specificity in development and control of the immune system and the discovery of the principle for production of monoclonal antibodies"

1986 First humanized antibodies approved by the US Food and Drug Administration (FDA).

1997 The first monoclonal antibody for cancer, Rituximab, approved by the FDA for treating non-Hodgkin's lymphoma.

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- Rituximab (anti-CD20) … 1997
  - LNH y LLC, enf. autoinmunes (AR)
- Trastuzumab (anti-HER2) … 1998
  - Cáncer de mama Her2/neu
- Cetuximab (anti-EGFR) … 2004 y 2006
  - Cáncer colorrectal, cabeza y cuello
- Bevacizumab (anti-VEGF) … 2004 a 2009
  - Cáncer colorrectal, cáncer de pulmón no microcítico, glioblastoma, cáncer de mama, carcinoma renal

Nomenclatura (DCI) de los anticuerpos monoclonales

RITUXIMAB

IBRITUMOMAB

OFATUMUMAB

OBINUTUZUMAB

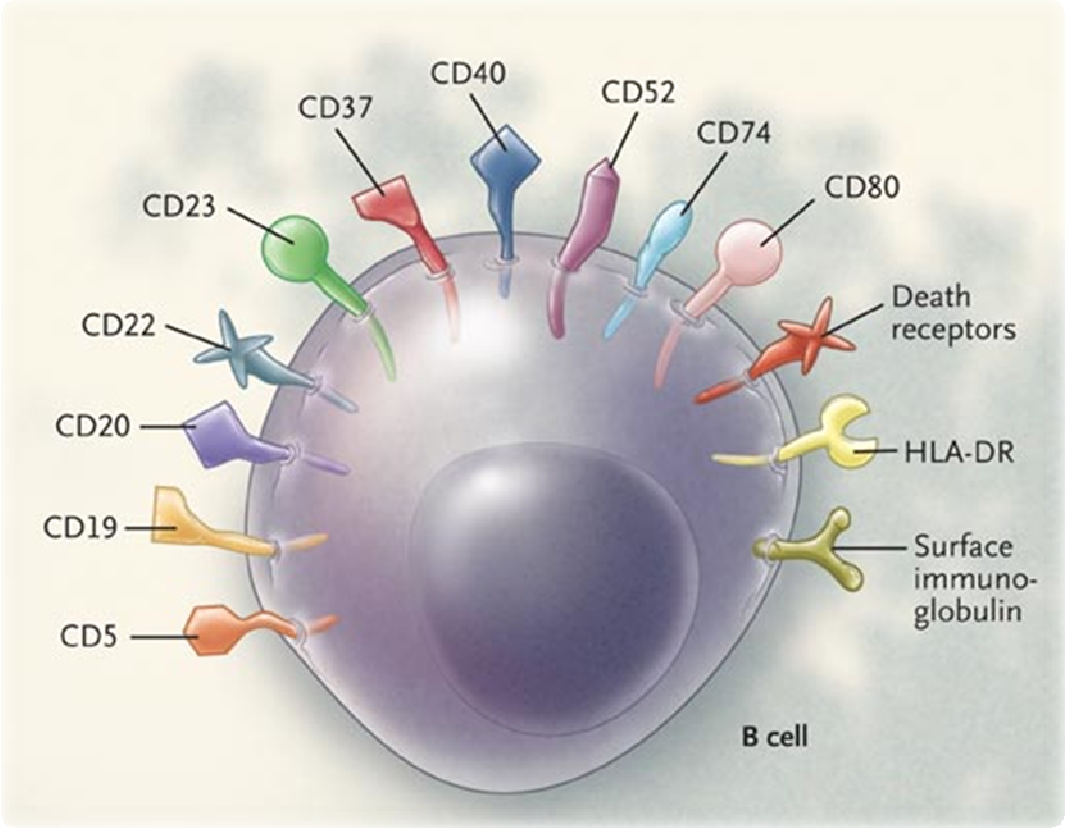
Nombre	Diana o área terapéutica	Origen	Tipo de anticuerpo
Elegido por el fabricante	*-anibi-: angiogénesis* *-ci-: cardiovascular* *-ki-: interleucinas* *-li: sistema inmune* *-tox-: toxina* *-tu-: tumores* *-vi-: virus*	*-xi-: quimérico* *-o:- murino* *-u-: humano* *-zu-: humanizado*	*-mab: monoclonal* *-pab: policlonal*

El sistema inmune como herramienta terapéutica

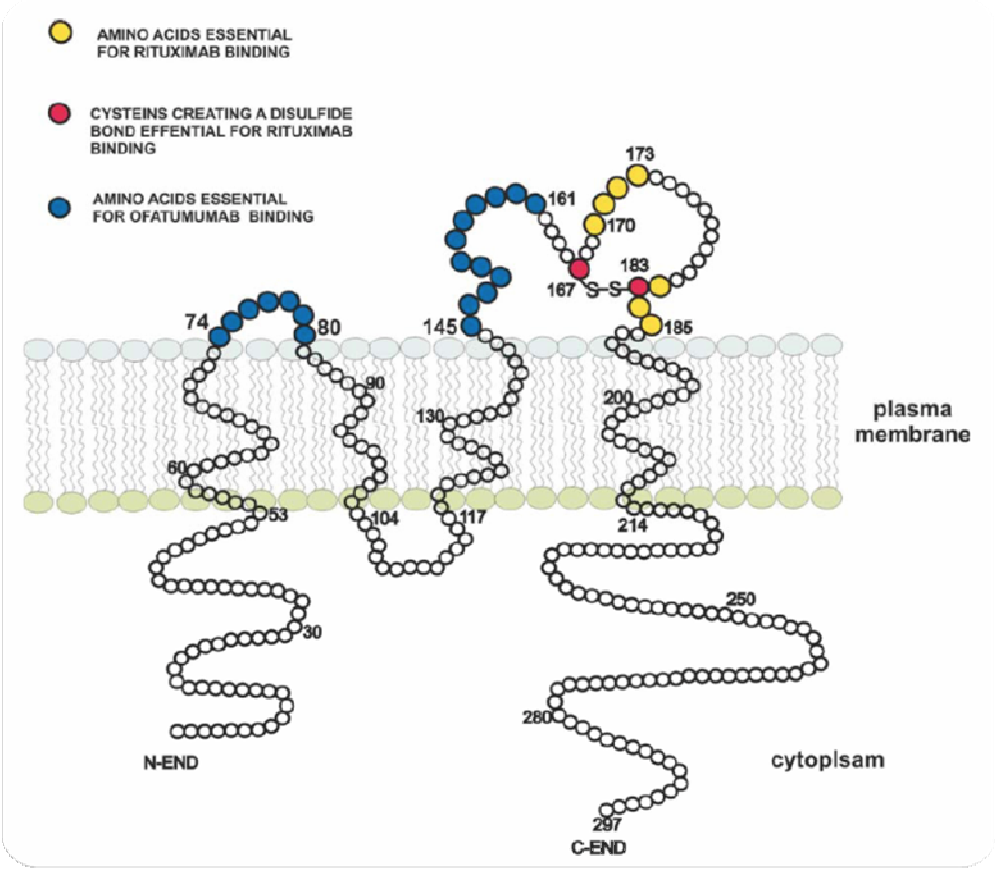
- Anticuerpos, anticuerpos, y más anticuerpos
  - El *surface-ome* como diana terapéutica
  - Anticuerpos conjugados a fármacos
  - Restaurar la vigilancia inmunológica antitumoral
- Inmunoterapia celular adoptiva
  - Trasplante alogénico
  - CARs (*Chimeric Antigen Receptors*)
- Vacunación anti-tumoral

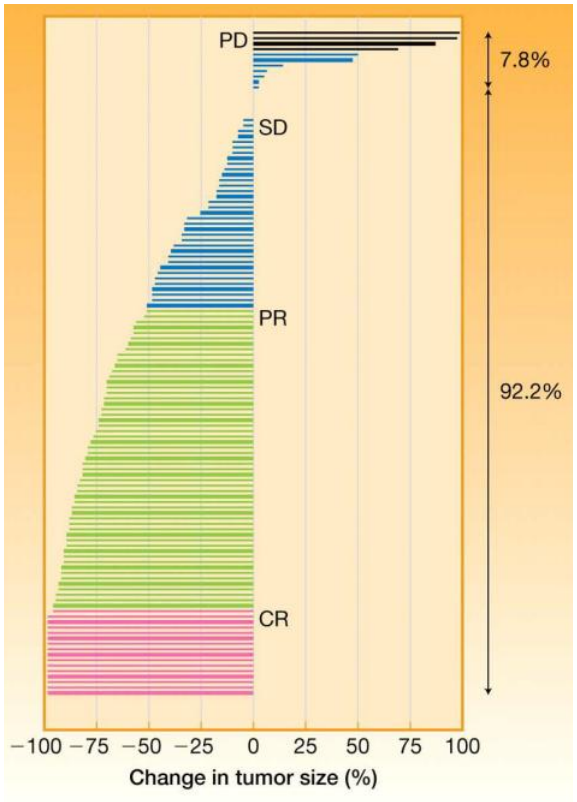
El *"surface-ome"* como diana terapéutica

Antígenos de superficie de las células B



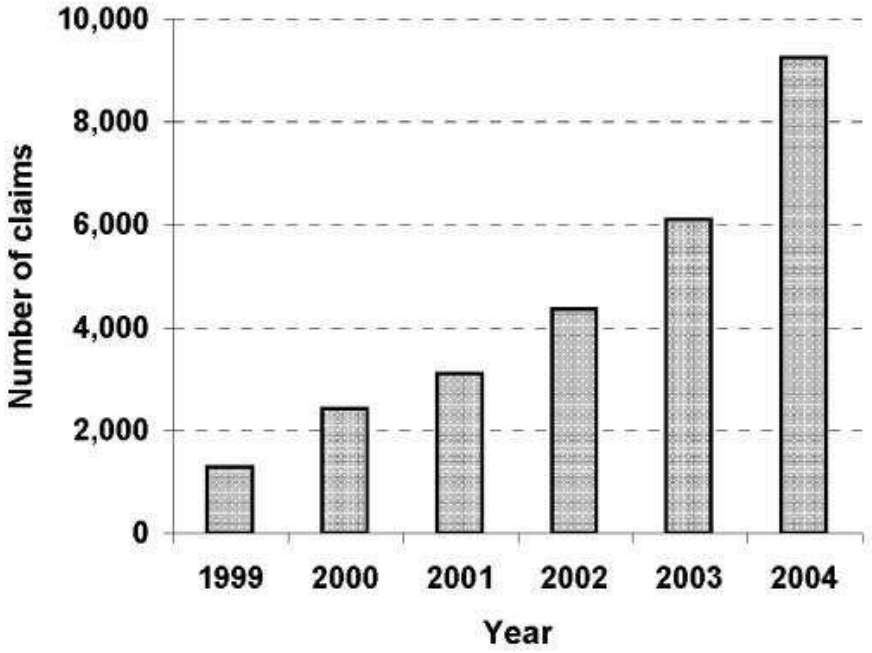
CD20 como diana terapéutica

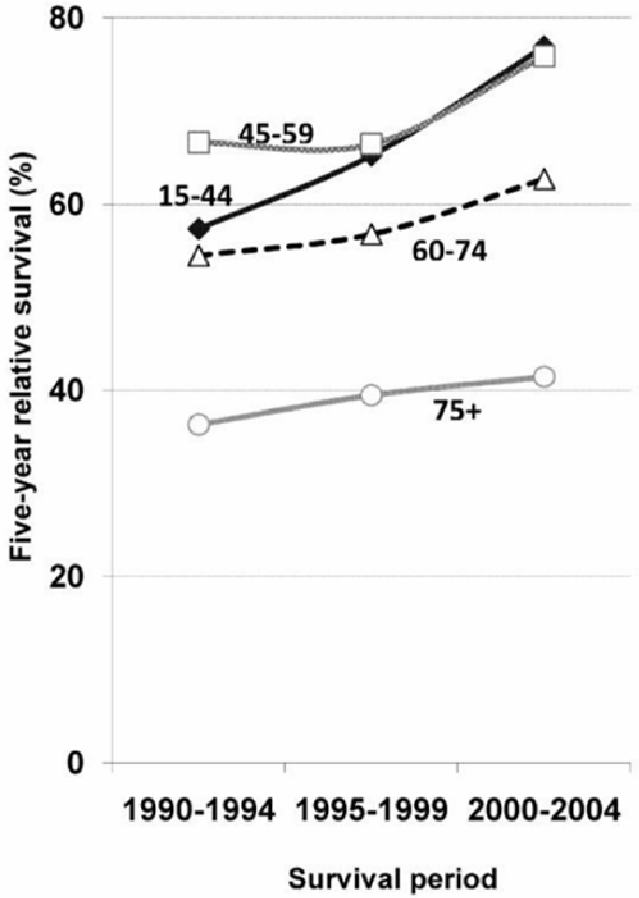




Aumento de la supervivencia de los linfomas no Hodgkin

Number of times rituximab was dispensed for treatment on NHL, NSW Australia, 1999-2004





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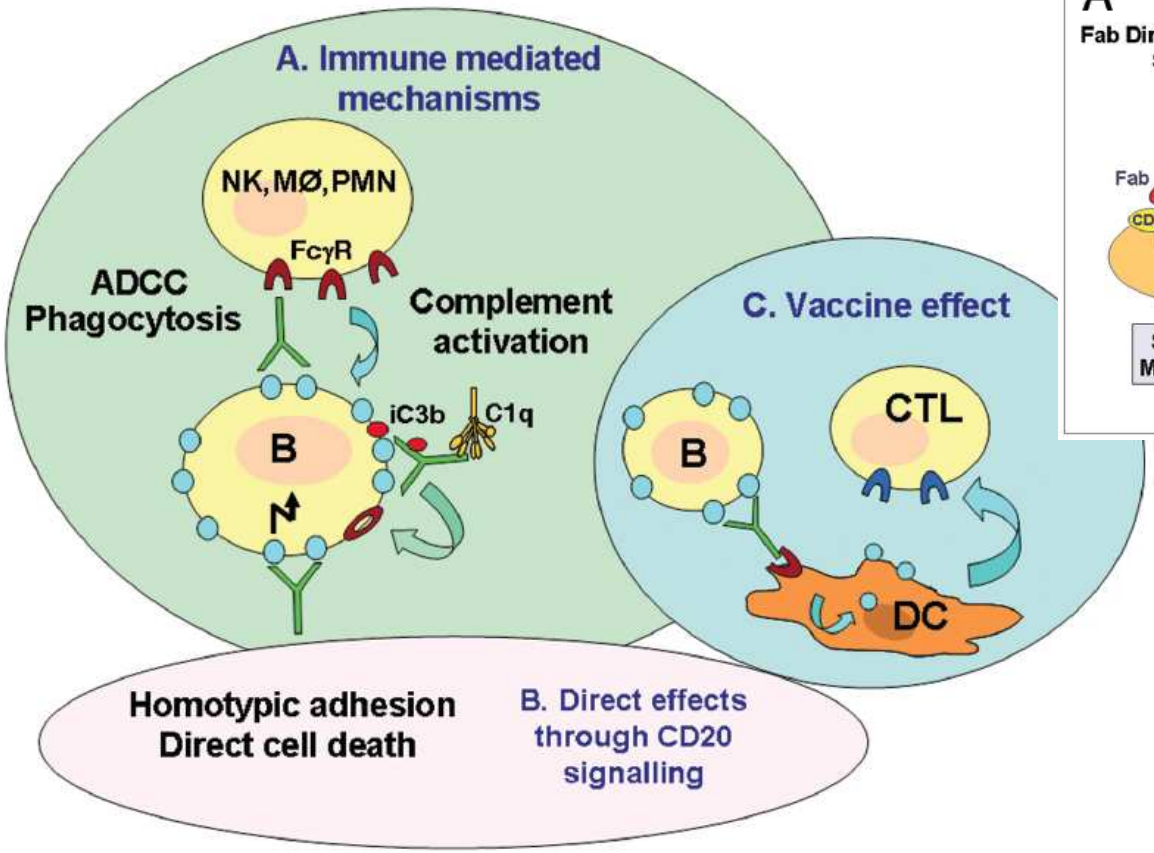
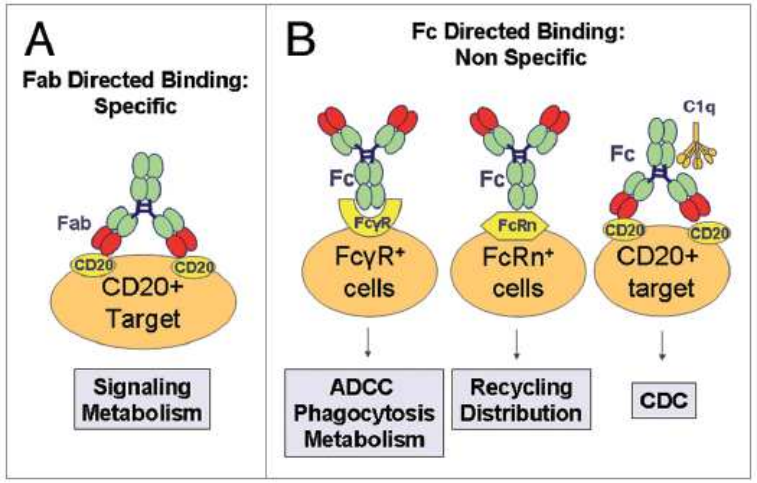
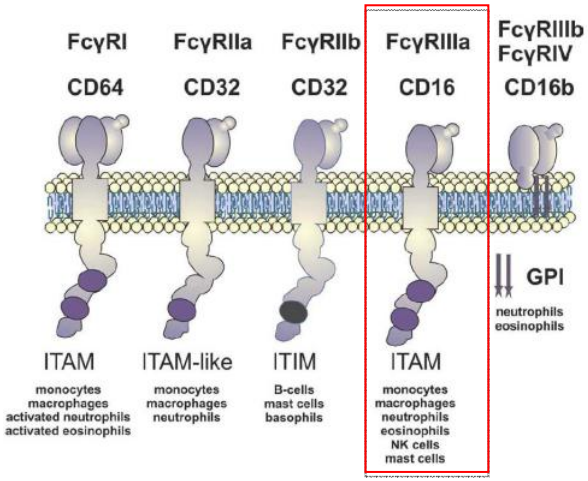
¿Por qué son tan eficaces los anticuerpos monoclonales anti-CD20?





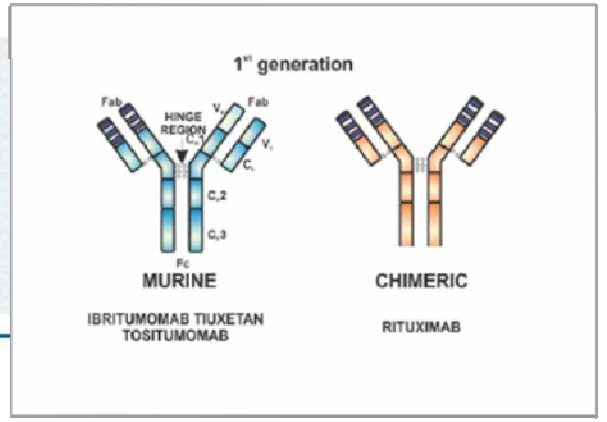


Modificado de Golay J, et al. mAbs 2013;5:1–12

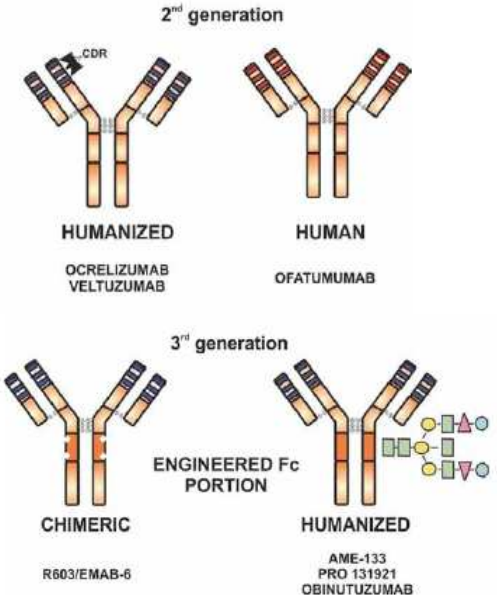
Eficacia de rituximab

Lymphoma Subtype	Prior Treatment	Treatment	Result	Reference
Follicular	No	R-CVP vs. CVP	Time to treatment failure, 27 mo vs. 7 mo; P<0.001	Marcus et al.[33]
Follicular	No	R-CHOP vs. CHOP	Treatment failure, 28/233 patients vs. 61/205 patients; P<0.001	Hiddemann et al.[34]
Follicular	Yes	R-CHOP vs. CHOP	Response rate, 85.1% vs. 72.3%; P<0.001	van Oers et al.[35]†
Follicular or mantle cell	Yes	R-FCM vs. FCM	Response rate, 79% vs. 58%; P=0.001	Forstpointner et al.[36]
Diffuse large B-cell	No	R-CHOP vs. CHOP	Median event-free survival, 3.8 yr vs. 1.1 yr; P<0.001	Coiffier et al.[37]
Diffuse large B-cell	No	R-CHOP vs. CHOP	3-yr event-free survival rate, 79% vs. 59%; P<0.001	Pfreundschuh et al.[38]
Diffuse large B-cell	No	R-CHOP vs. CHOP	3-yr failure-free survival rate, 54% vs. 46%; P=0.04	Habermann et al.[39]
Mantle cell	No	R-CHOP vs. CHOP	Median time to treatment failure, 21 mo vs. 14 mo; P=0.01	Lenz et al.[40]
Follicular	No	R-MCP vs. MCP	4-yr overall survival rate, 87% vs. 74%; P=0.01	Herold et al.[41]

Building up from the old



1. La importancia del epítope
   - Segunda generación de anticuerpos humanizados
     - Ocrelizumab
     - Ofatumumab
2. Glicoingeniería
   - Obinutuzumab (GA101)
   - Anticuerpos monoclonales *"bio-better"* (GS4:0)
3. Mejorando el cumplimiento terapéutico
   - Administración subcutánea (anticuerpos + rHuPH20)



Epitope binding does matter

Type I antibodies	Type II antibodies
Class I epitope	Class II epitope
Localize CD20 to lipid rafts	Do not localize CD20 to lipid rafts
High CDC	Low CDC
ADCC activity	ADCC activity
Full binding capacity	Half binding capacity
Weak homotypic aggregation	Homotypic aggregation
Cell death induction	Stronger cell death induction
Rituximab, ocrelizumab (2H7), ofatumumab (2F2)	GA101, tositumomab (B1)

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Klein C, et al. mAbs 2013;5:22–33

Ofatumumab Is More Efficient than Rituximab in Lysing B Chronic Lymphocytic Leukemia Cells in Whole Blood and in Combination with Chemotherapy

Luca Bologna, Elisa Gotti, Fabio Da Roit, Tamara Intermesoli, Alessandro Rambaldi, Martino Introna, and Josée Golay

Ofatumumab (OFA) is a human anti-CD20 Ab approved for treatment of fludarabine-refractory B chronic lymphocytic leukemia (B-CLL). The efficacy of different immunotherapeutic strategies is best investigated in conditions that are as physiologic as possible. We have therefore compared the activity OFA and rituximab (RTX), alone or in combination with chemotherapeutic agents in unmanipulated whole blood assays, using flow cytometry. OFA (10–100 μg/ml) lysed B-CLL targets in whole blood more efficiently and with faster kinetics than RTX, with a mean 56% lysis at 24 h compared with 16%. This activity of OFA was fully complement dependent, as shown by >99% inhibition by anti-C5 Ab eculizumab and a lack of NK cell activation in whole blood. OFA-mediated NK cell activation was blocked by complement. OFA-mediated lysis could be increased an additional 15% by blocking CD55 and CD59 complement inhibitors. Interestingly, OFA-mediated lysis correlated significantly with CD20 expression levels (r^2 = 0.79). OFA showed overlapping dose response curves similar to those for RTX in phagocytosis assays using either human macrophages or neutrophils. However, phagocytosis was inhibited in the presence of serum or whole blood. Finally, combined treatment with mafosfamide and fludarabine showed that these therapeutic drugs are synergistic in B-CLL whole blood assays and show superior activity when combined with OFA compared with RTX. These results confirm in B-CLL samples and in physiologic conditions the superior complement mediated cytotoxicity induced by OFA alone compared with RTX, the lack of NK cell activation, and phagocytosis in these conditions and suggest effective chemoimmunotherapy strategies using this new generation anti-CD20 Ab. *The Journal of Immunology*, **2013, 190: 231–239.**

Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell–mediated B-cell cytotoxicity

Ekkehard Mössner,[1] Peter Brünker,[1] Samuel Moser,[1] Ursula Püntener,[1] Carla Schmidt,[1] Sylvia Herter,[1] Roger Grau,[1] Christian Gerdes,[1] Adam Nopora,[1] Erwin van Puijenbroek,[1] Claudia Ferrara,[1] Peter Sondermann,[1] Christiane Jäger,[1] Pamela Strein,[2] Georg Fertig,[2] Thomas Friess,[2] Christine Schüll,[2] Sabine Bauer,[2] Joseph Dal Porto,[3] Christopher Del Nagro,[3] Karim Dabbagh,[3] Martin J. S. Dyer,[4] Sibrand Poppema,[5] Christian Klein,[1] and Pablo Umaña[1]

[1]GlycArt Biotechnology AG, Schlieren, Switzerland; [2]Discovery Oncology, Roche Diagnostics GmbH, Penzberg, Germany; [3]Discovery Inflammation, Roche Glycart, Palo Alto, CA; [4]MRC Toxicology Unit, University of Leicester, Leicester, United Kingdom; and [5]Medical Center, University of Groningen, Groningen, The Netherlands

CD20 is an important target for the treatment of B-cell malignancies, including non-Hodgkin lymphoma as well as autoimmune disorders. B-cell depletion therapy using monoclonal antibodies against CD20, such as rituximab, has revolutionized the treatment of these disorders, greatly improving overall survival in patients. Here, we report the development of GA101 as the first Fc-engineered, type II humanized IgG1 antibody against CD20. Relative to rituximab, GA101 has increased direct and immune effector cell-mediated cytotoxicity and exhibits superior activity in cellular assays and whole blood B-cell depletion assays. In human lymphoma xenograft models, GA101 exhibits superior antitumor activity, resulting in the induction of complete tumor remission and increased overall survival. In nonhuman primates, GA101 demonstrates superior B cell–depleting activity in lymphoid tissue, including in lymph nodes and spleen. Taken together, these results provide compelling evidence for the development of GA101 as a promising new therapy for the treatment of B-cell disorders. (*Blood*. 2010;115(22): 4393-4402)

Glycoengineered CD20 antibody obinutuzumab activates neutrophils and mediates phagocytosis through CD16B more efficiently than rituximab

Josée Golay,[1] Fabio Da Roit,[1] Luca Bologna,[1] Claudia Ferrara,[2] Jeanette H. Leusen,[3] Alessandro Rambaldi,[1] Christian Klein,[2] and Martino Introna[1]

[1]Center of Cellular Therapy "G. Lanzani," Division of Haematology, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy; [2]Roche Glycart AG, Roche Pharma Research and Early Development, Schlieren, Switzerland; and [3]Immunotherapy Laboratory, Department of Immunology, University Medical Center, Utrecht, The Netherlands

Key Points

- Phagocytosis of CLL targets by neutrophils is a novel mechanism of action of the glycoengineered anti-CD20 antibody obinutuzumab.
- This mechanism takes place in physiological conditions and requires CD16B and CD32A.

Obinutuzumab (GA101) is a glycoengineered type 2 CD20 antibody with enhanced CD16A-binding and natural killer–mediated cytotoxicity. CD16B is highly homologous to CD16A and a major FcγR on human polymorphonuclear neutrophils (PMNs). We show here that glycoengineered obinutuzumab or rituximab bound CD16B with approximately sevenfold higher affinity, compared with nonglycoengineered wild-type parental antibodies. Furthermore, glycoengineered obinutuzumab activated PMNs, either purified or in chronic lymphoblastic leukemia whole blood, more efficiently than wild-type rituximab. Activation resulted in a 50% increase in CD11b expression and 70% down-modulation of CD62L on neutrophils and in release of tumor necrosis factor alpha, IL-6, and IL-8. Activation was not accompanied by generation of reactive oxygen species or antibody-dependent cellular cytotoxicity activity, but led to up to 47% phagocytosis of glycoengineered anti-CD20 opsonized chronic lymphoblastic leukemia targets by purified PMNs. Significant phagocytosis was observed in whole blood, but only in the presence of glycoengineered antibodies, and was followed by up to 50% PMN death. Finally we show, using anti-CD16B and anti-CD32A Fab and $F(ab')_2$ fragments, that both of these receptors are involved in PMN activation, phagocytosis, and cell death induced by glycoengineered antibodies. We conclude that phagocytosis by PMNs is an additional mechanism of action of obinutuzumab mediated through its higher binding affinity for CD16B. (*Blood*. 2013;122(20):3482-3491)

Eficacia de ofatumumab y obinutuzumab en pacientes con LLC

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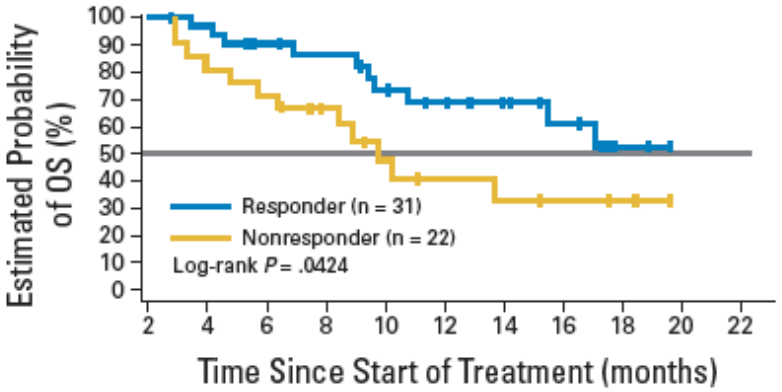

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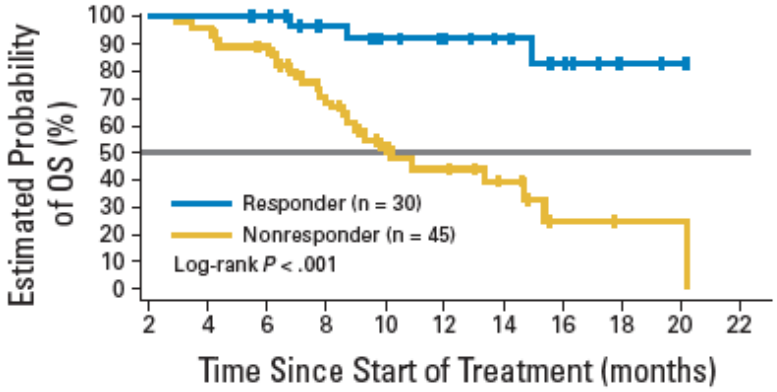

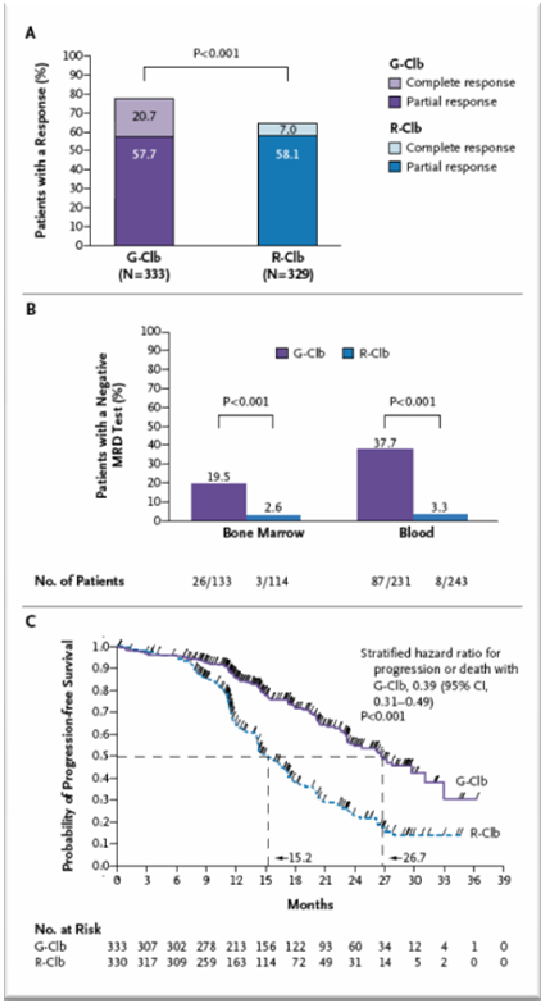

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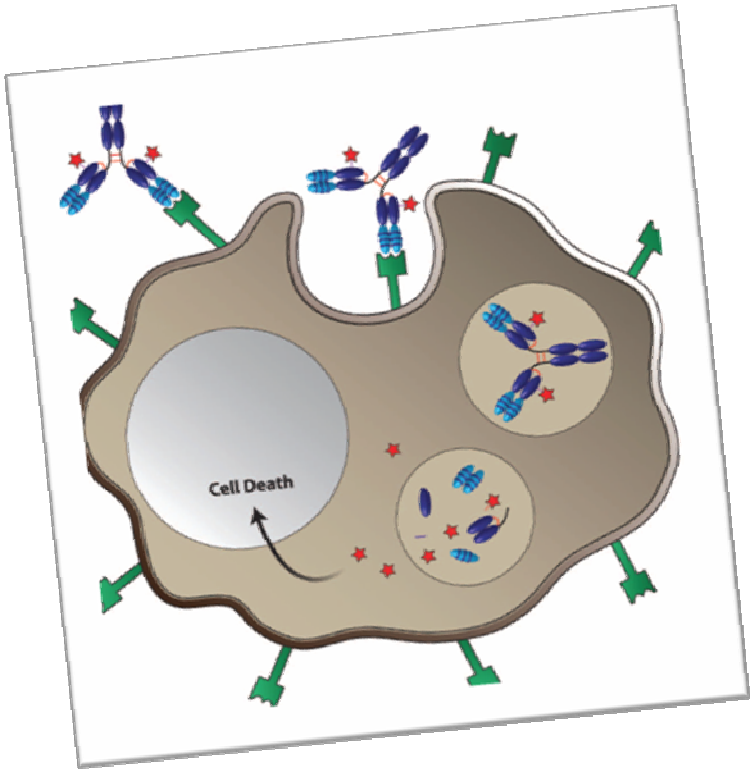
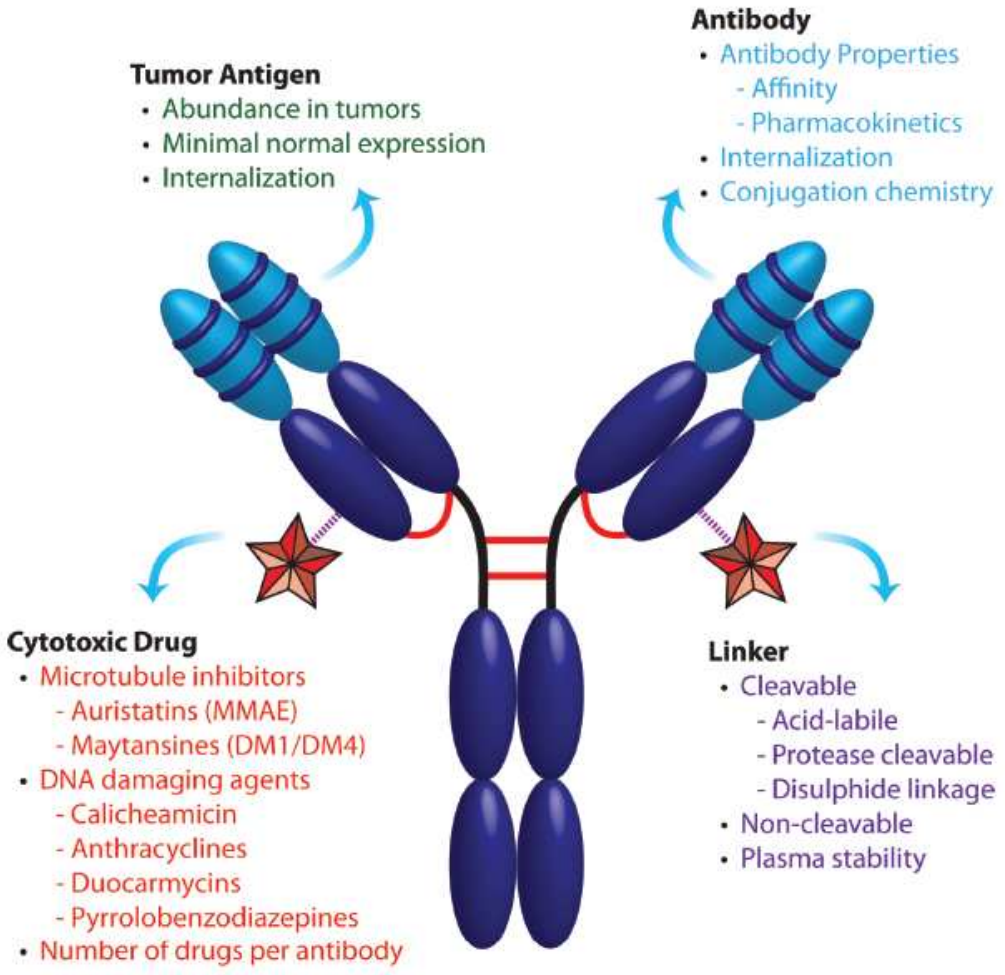
Anticuerpos monoclonales no conjugados

Antibody	Target
Rituximab	CD20
Alemtuzumab	CD52
Human or humanized anti-CD20 antibodies	CD20
Lumiliximab	CD23
Anti-TRAIL	DRD
Bevacizumab	VEGF
Galiximab	CD80
Epratuzumab	CD22
SGN-40	CD40
Anti-CD74	CD74

Anticuerpos conjugados a fármacos (ADC)





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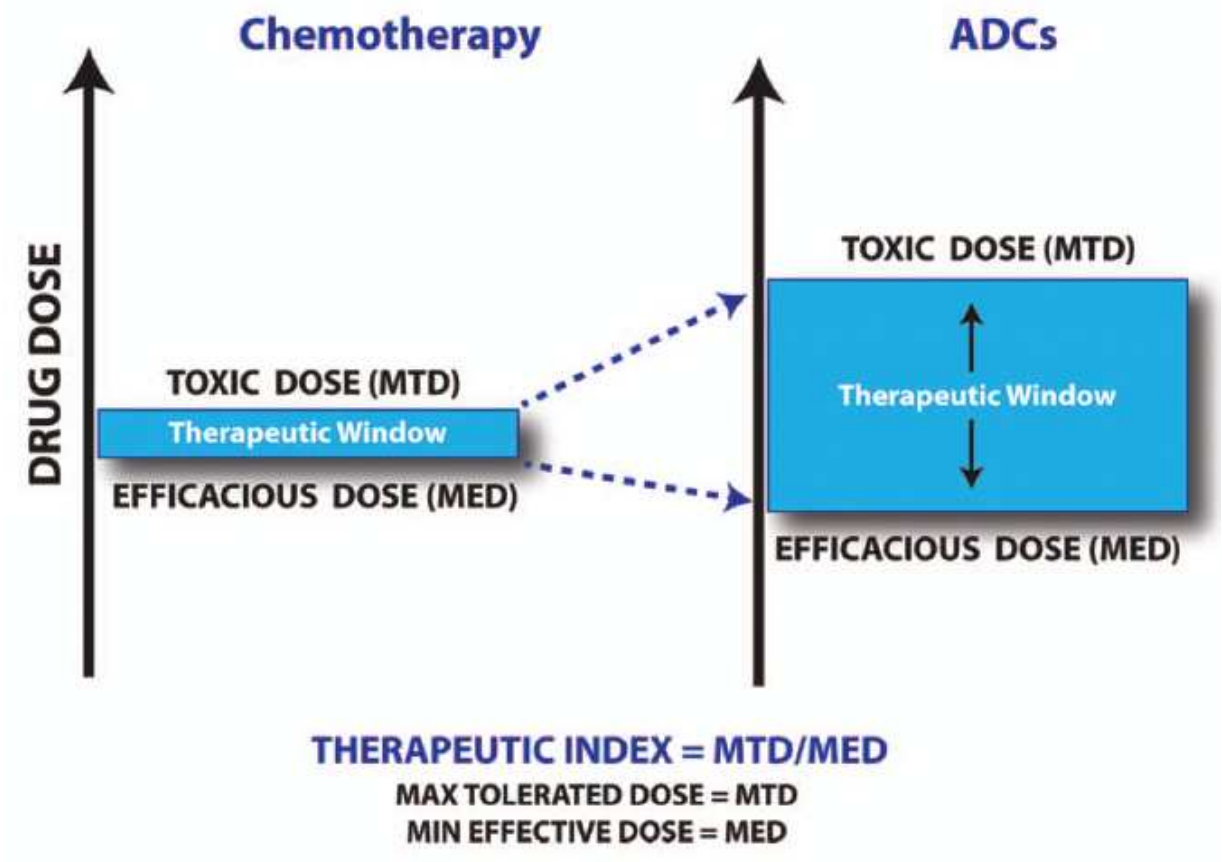
Panowski S, et al. mAbs 2014;6:34–45

Ventajas de ADC para “liberar” citotóxicos frente a la administración tradicional



Panowski S, et al. mAbs 2014;6:34–45

Anticuerpos monoclonales conjugados

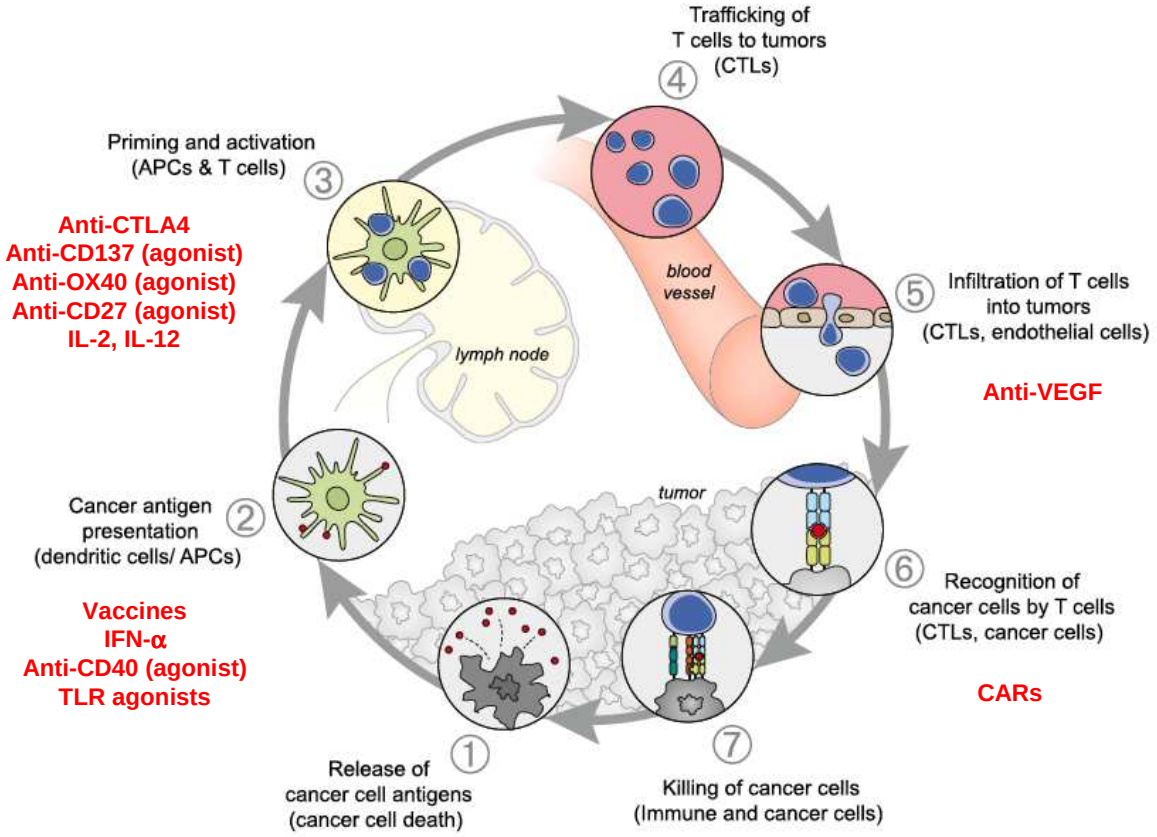
ADC	Diana	Agente citotóxico
Inotuzumab ozagamicin	CD22	Calicheamicin
Coltuximab ravtansine (SAR3419)	CD19	DM4
Pinatuzumab vedotina	CD22	MMAE
Polatuzumab vedotina	CD79b	MMAE
Brentuximab vedotina	CD30	MMAE

DM4: Maytansines; MMAE: monomethyl auristatin E

Las células T como terapia antitumoral

- Trasplante alogénico
  - *Weiden PL, et al. NEJM 1979;300:1068*
- Infusión de linfocitos del donante
  - *Kol HJ, et al. Blood 1990;76:2462*
- Vacuna anti-tumoral (Sipuleucel-T, Provenge®)
  - *Kantoff PW, et al. NEJM 2010;363:411*
- Ipilimumab, Nivolumab
  - *Hodi S, et al. NEJM 2010;363:711*
  - *Wolchok JD, et al. NEJM 2013;369:122*
- Inmunoterapia celular adoptiva, CARs (*Chimeric Antigen Receptors*)
  - *Porter DL, et al. NEJM 2011;365:725*

El ciclo de la inmunidad tumoral



Modificado de Chen & Mellman. Immunity 2013;39:1–10

Restaurando la inmunidad antitumoral: anticuerpos frente a PD-1

Los tumores son antigénicos, pero poco inmunogénicos

¿Cómo hacer que un tumor sea menos "inmunotolerable"?

Aumentando la respuesta autóloga de células T

Mediante aumento expresión de Ags asociados a leucemia

Interferón

Mediante manipulación *ex vivo* de células T

- ✓ **Inmunoterapia adoptiva**
- ✓ **CARs**
- ✓ **Vacunas**

Mediante manipulación farmacológica de moléculas co-estimuladoras de céls. T

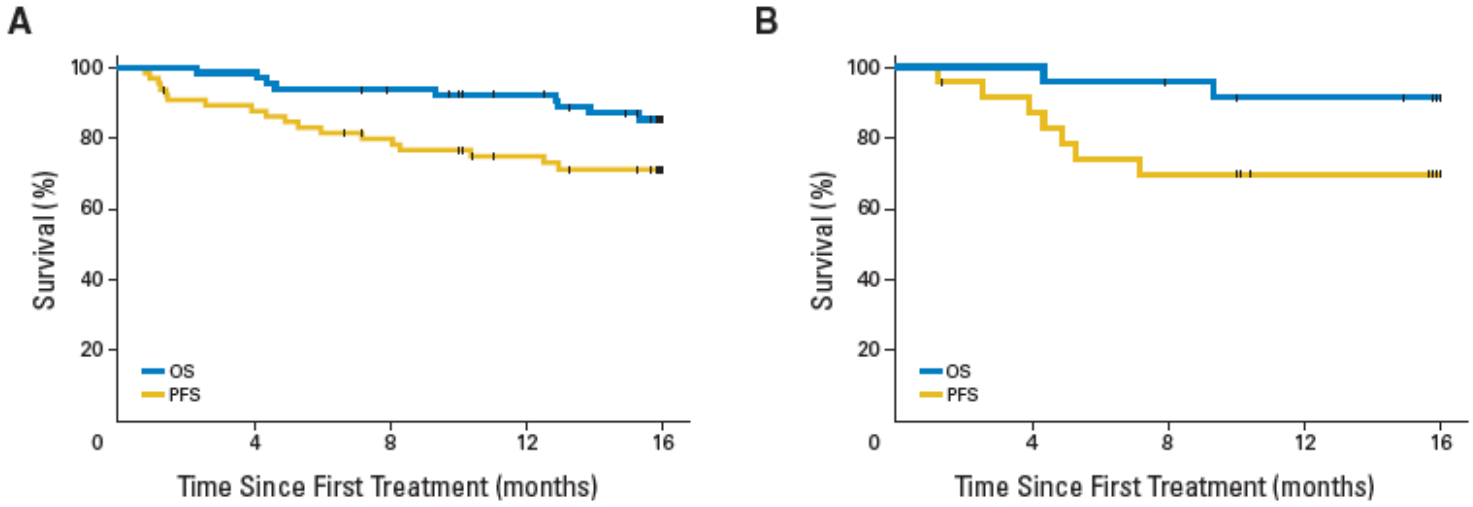
Ipilimumab
Nivolumab

Desarrollo clínico de los inhibidores de PD-1 y PD-L1

Target	Antibody	Molecule	Development stage
PD-1	Nivolumab (BMS-936558)	Fully human IgG4	Phase III multiple tumors (melanoma, RCC, NSCLC, HNSCC)
	Pembrolizumab (MK-3475)	Humanized IgG4	Phase I-II multiple tumors Ph III NSCLC/melanoma
	Pidilizumab (CT-011)	Humanized IgG1	Phase II multiple tumors
PD-L1	MEDI-4736	Engineered human IgG1	Phase I-II multiple tumors
	MPDL-3280A	Engineered human IgG1	Phase I-II multiple tumors Phase III NSCLC
	MSB0010718C	Fully human IgG1	Phase I solid tumors

Pidilizumab en la práctica clínica

- Pidilizumab 1.5 mg/kg every 42 days for 3 cycles, beginning 30 to 90 days from AHSCT



Armand P, et al. J Clin Oncol 2013;31:4199–206

SERUM
Behring
Phenol

¿Cómo difiere la formulación de rituximab SC de la formulación IV?

- La formulación de rituximab SC contiene el mismo anticuerpo, concentrado 12 veces para reducir el volumen (5-12 mL)

- La administración SC es posible, mediante un sistema de liberación enzimática (Halozyme)
  - La co-formulación con hialuronidasa humana recombinante (rHuPH20) permite administración de volúmenes considerados demasiado grandes para su administración SC

- Empleo de dosis fija, en lugar de dosis por superficie corporal

Cuestiones planteadas en el programa de desarrollo clínico de rituximab SC

- Programa clínico diseñado para asegurar la no inferioridad de la exposición a la molécula activa (mediante la determinación de parámetros farmacocinéticos) en la formulación SC

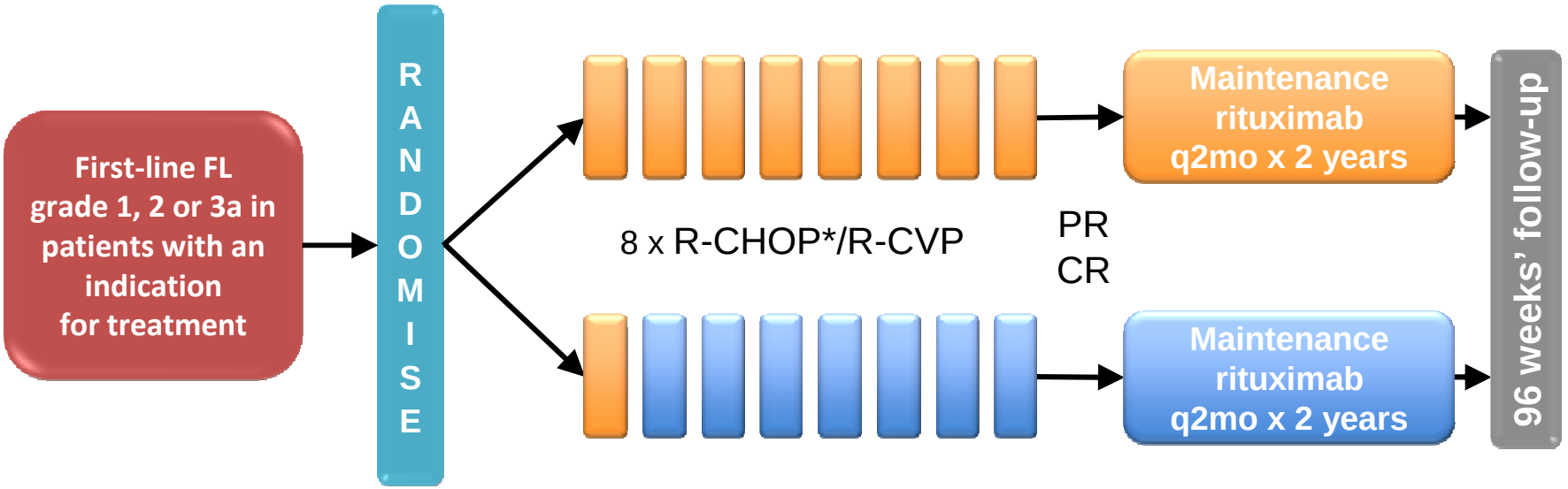
  - ¿Es posible su desarrollo mediante el empleo de dosis fija en lugar de la dosis calculada por superficie corporal?
  - ¿Es posible mantener la misma eficacia y seguridad?

Programa de desarrollo clínico de rituximab SC

Key studies – dose-finding and confirmation

Study	Objective 1	Objective 2
BP22333 Phase Ib SparkThera	Identify a fixed dose in FL maintenance (Stage 1: dose finding)	Confirm the fixed dose (Stage 2: dose confirmation)
BO22334 Phase III SABRINA	Confirm the fixed dose in FL induction with chemotherapy followed by maintenance therapy (Stage 1)	Confirm that the change of administration route does not compromise the proven efficacy of rituximab (Stage 2)
BO25341 Phase Ib/III SAWYER	Identify a fixed dose in CLL (Stage 1: dose finding)	Confirm the fixed dose (Stage 2: dose confirmation)

Estudio SABRINA: Eficacia y seguridad de rituximab SC



Davies A, et al. Br J Haematol 2013;161(suppl 1): Abstract 15

Estudio SABRINA: perfil de seguridad comparable de la formulación SC

Patients, n (%)	CT + Rituximab IV	CT + Rituximab SC
Any-grade AE	57 (88)	57 (92)
Serious AE	14 (22)	14 (23)
Any-grade treatment-related AE	30 (46)	45 (73)
ARRs (>90% grade ½)	21 (32)	31 (50)
Grade ≥ 3 AE	30 (46)	29 (47)
Grade ¾ neutropenia	14 (22)	16 (26)
Febrile neutropenia	2 (3)	6 (10)

ARR = administration-related reactions

Davies A, et al. Br J Haematol 2013;161(suppl 1): Abstract 15

Estudio SABRINA: efectos adversos según superficie corporal

		Rituximab IV		Rituximab SC	
		n	Patients with AEs, n (%)	n	Patients with AEs, n (%)
SAEs	All	65	14 (22)	62	14 (23)
	Low BSA	16	3 (19)	26	5 (19)
	Medium BSA	27	9 (33)	15	7 (47)
	High BSA	21	2 (10)	21	2 (10)
Grade ≥3 AEs	All	65	30 (46)	62	29 (47)
	Low BSA	16	8 (50)	26	15 (58)
	Medium BSA	27	15 (56)	15	7 (47)
	High BSA	21	7 (33)	21	7 (33)

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Davies A, et al. Haematologica 2013;98(suppl): Abstract P308

Estudio SABRINA: eficacia comparable de la formulación SC

	Investigator-assessed		Independent review	
	IV (n = 64)	SC (n = 63)	IV (n = 64)	SC (n = 63)
Overall response rate, n (%)	54 (84.4)	57 (90.5)	56 (87.5)	54 (85.7)
CR/CRu, n (%)	19 (29.7)	29 (46.0)	12 (18.8)	17 (27.0)
PR, n (%)	35 (54.7)	28 (44.4)	44 (68.8)	37 (58.7)
Stable disease, n (%)	3 (4.7)	2 (3.2)	1 (1.6)	4 (6.3)
Progressive disease, n (%)	1 (1.6)	0 (0)	0 (0)	2 (3.2)
Missing/invalid, n (%)	6 (9.4)	4 (6.3)	7 (10.9)	3 (4.8)

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Estudio SABRINA, etapa 1: respuesta según el régimen de inducción

		Rituximab IV		Rituximab SC	
		n	Responders, n (%)	n	Responders, n (%)
ORR	All patients	64	54 (84.4)	63	57 (90.5)
	CHOP	40	34 (85.0)	40	37 (92.5)
	CVP	24	20 (83.3)	23	20 (87.0)
CR/CRu	All patients	64	19 (29.7)	63	29 (46.0)
	CHOP	40	13 (32.5)	40	17 (42.5)
	CVP	24	6 (25.0)	23	12 (52.2)

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Estudio SABRINA, etapa 1: respuesta según superficie corporal

		Rituximab IV		Rituximab SC	
		n	Responders, n (%)	n	Responders, n (%)
ORR	All patients	64	54 (84.4)	63	57 (90.5)
	Low BSA (< 1.7 m^2)	16	15 (93.8)	26	22 (84.6)
	Medium BSA (1.7-1.9 m^2)	26	20 (76.9)	16	15 (93.8)
	High BSA (> 1.9 m^2)	21	18 (85.7)	21	20 (95.2)
CR/CRu	All patients	64	19 (29.7)	63	29 (46.0)
	Low BSA (< 1.7 m^2)	16	5 (31.3)	26	14 (53.8)
	Medium BSA (1.7-1.9 m^2)	26	7 (26.9)	16	8 (50.0)
	High BSA (> 1.9 m^2)	21	7 (33.3)	21	7 (33.3)

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Conclusiones

- La inmunoterapia con anticuerpos monoclonales ha cambiado la historia natural de los síndromes linfoproliferativos
- Los avances con los nuevos anticuerpos anti-CD20 se centran en mejorar el mecanismo de acción
  - Los nuevos anticuerpos conjugados a fármacos y la restauración de la inmunidad antitumoral ofrecen alternativas interesantes de futuro
- Nuevas formas de administración (SC) son técnicamente posibles, más convenientes para el paciente, mantienen la eficacia y seguridad y son coste-efectivas