Antibody Based Immunotherapy

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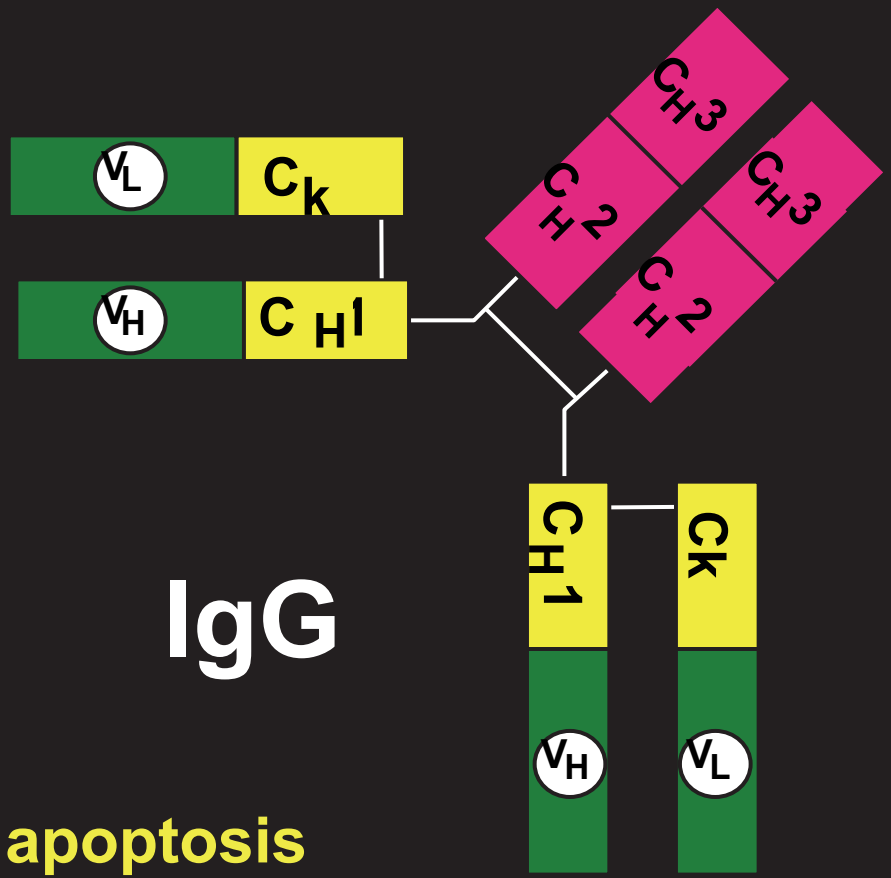
Disclosures: Co-inventor on NIH patent for moxetumomab pasudotox

sitc

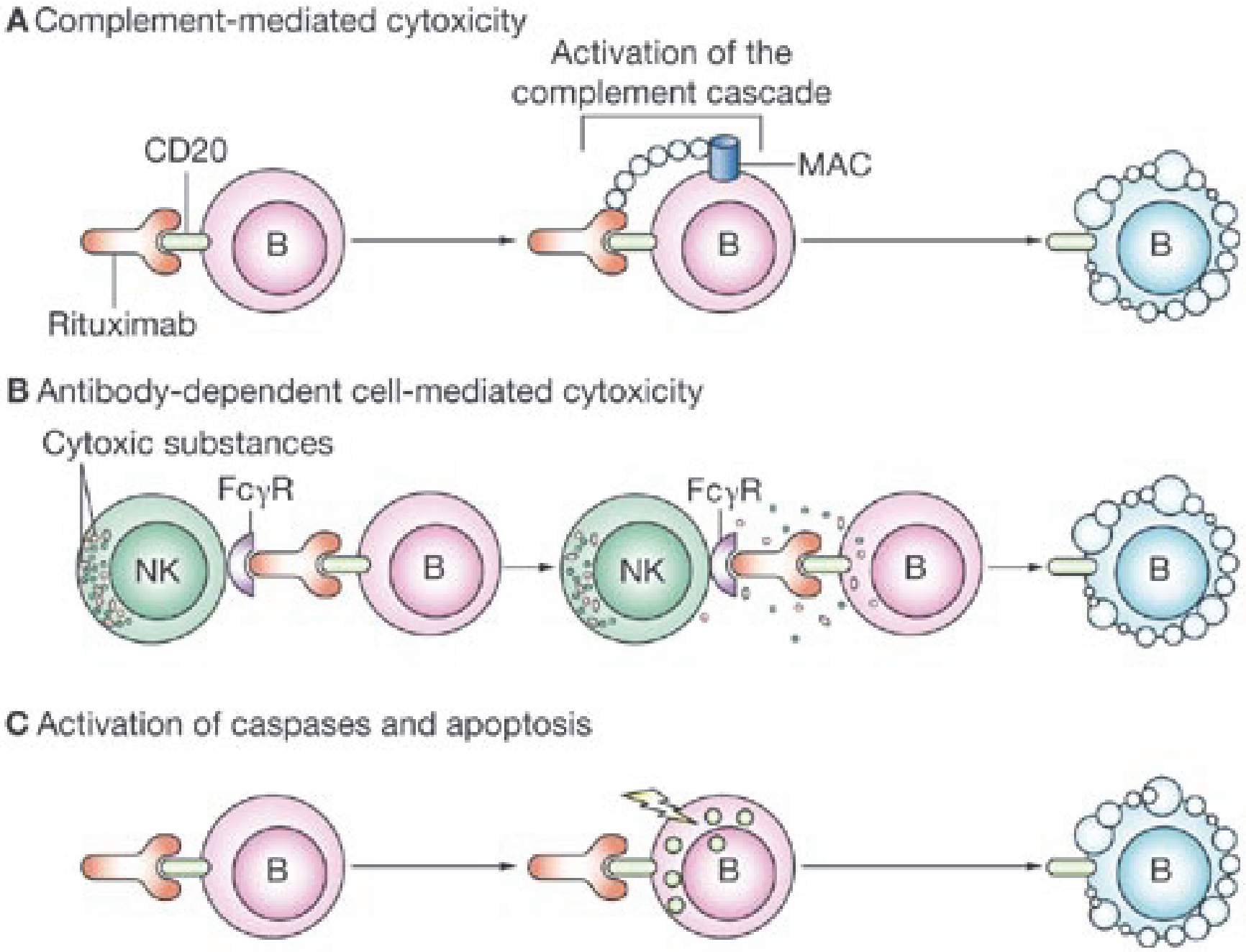
Society for Immunotherapy of Cancer

Monoclonal antibodies



IgG

Induce apoptosis
CDC
ADCC



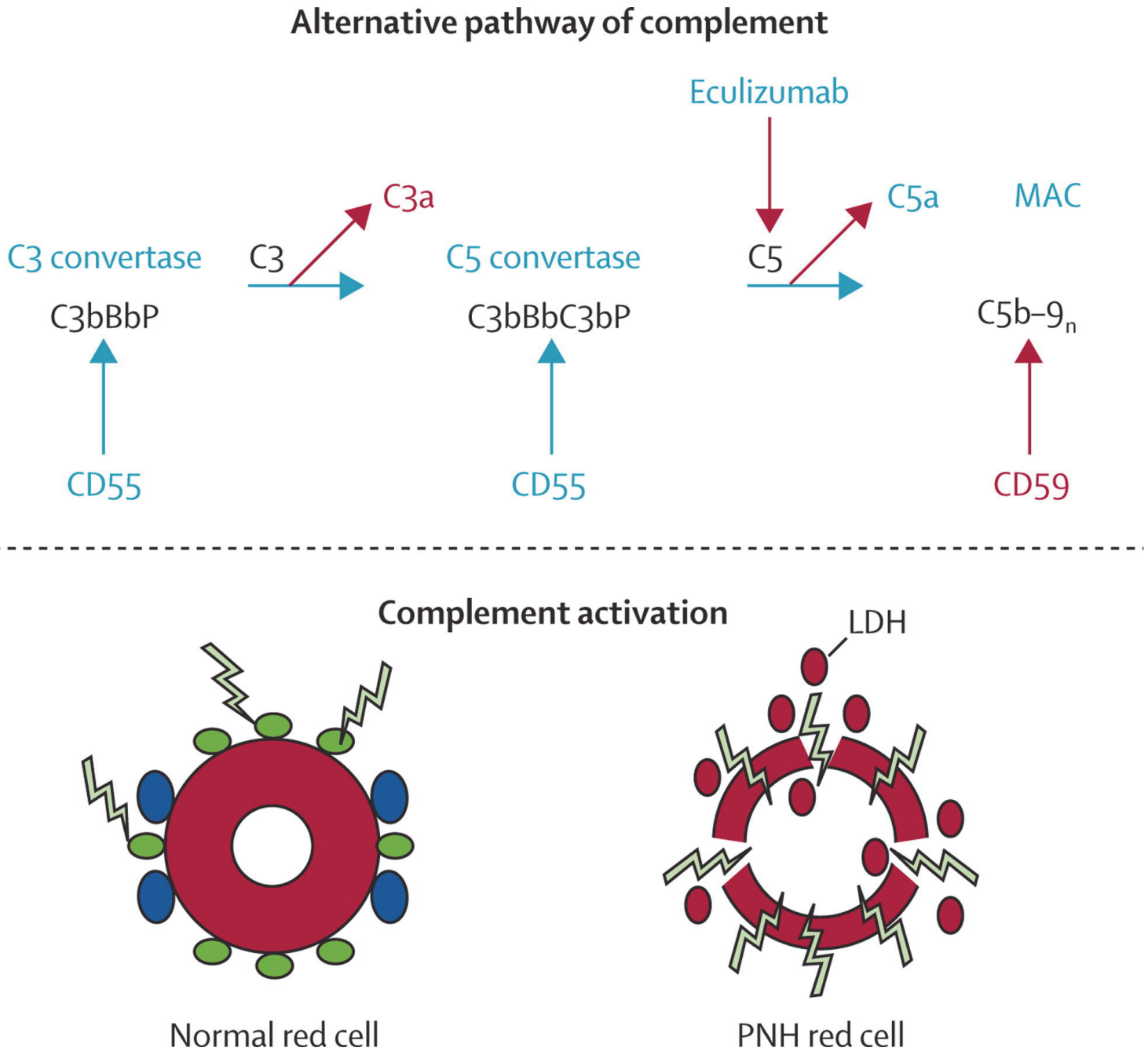
Bayry et al., Nature Clin Prac Rheum, 3:262-272

Unlabeled MAbs approved for malignancies

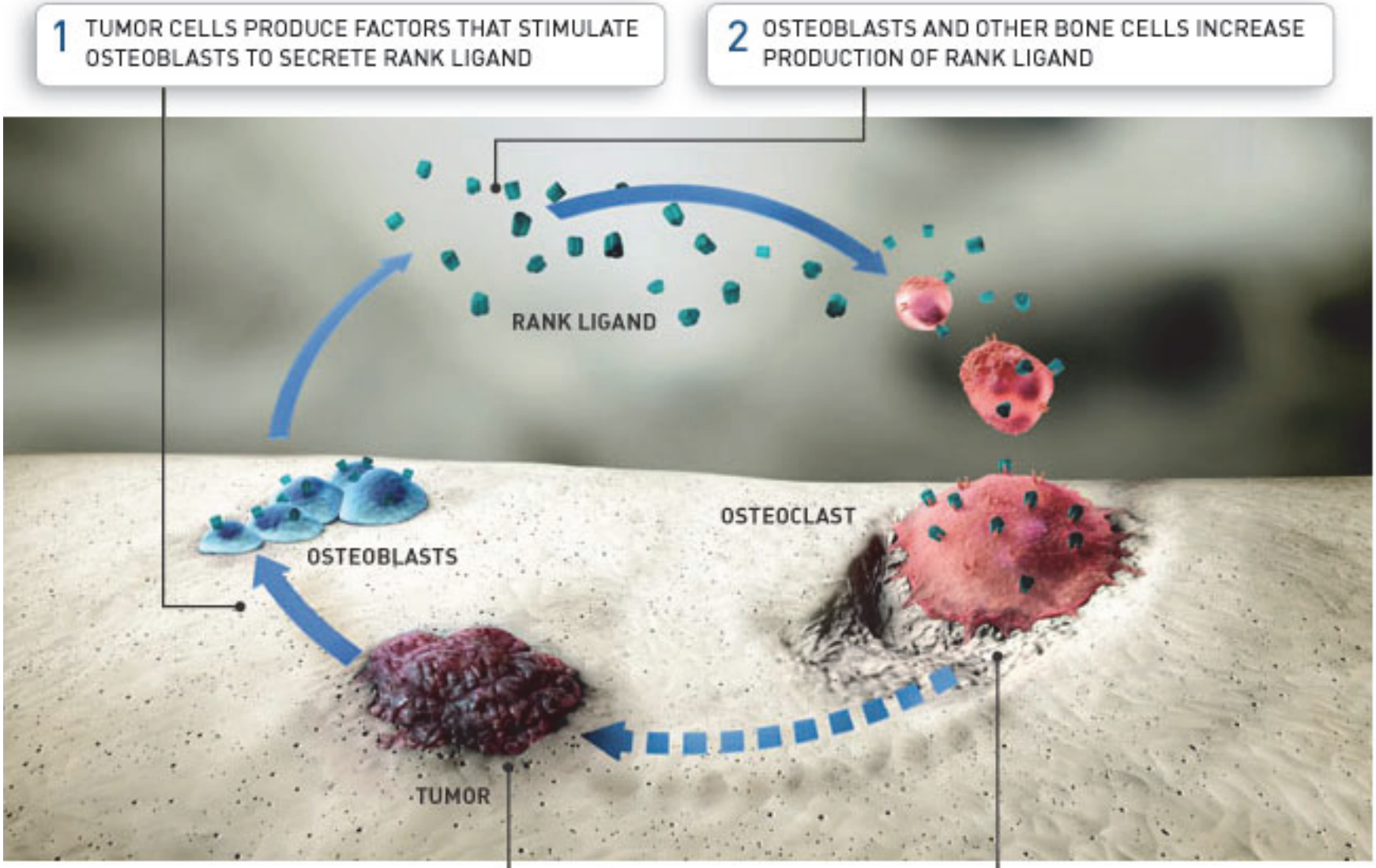
11/97	Rituximab	CD20	Non-Hodgkin's lymphoma (NHL)
10/98	Trastuzumab	Her2	Metastatic breast cancer (Gastric 10/10)
5/01	Alemtuzumab	CD52	B-cell chronic lymphocytic leukemia (CLL)
2/04	Bevacizumab	VEGF	Metastatic colorectal cancer (RCCA 7/09)
2/04	Cetuximab	EGFR	Metastatic colorectal cancer (EGFR+)
9/06	Panitumumab	EGFR	Colorectal cancer
3/07	Eculizumab	C5	Paroxysmal nocturnal hemoglobinuria (HUS 9/11)
10/09	Ofatumumab	CD20	CLL
11/10	Denosumab	RANKL	Prevention of SREs from bone metastases
3/11	Ipilimumab	CTLA4	Melanoma

Normal RBCs: glycosyl phosphatidylinositol (GPI)-anchored proteins decay accelerating factor [DAF or CD55] and membrane inhibitor of reactive lysis [MIRL or CD59]).

PNH cells express transmembrane proteins normally, but GPI-anchored proteins are not expressed because of mutant phosphatidyl-inositol glycan complementation class A (PIGA).

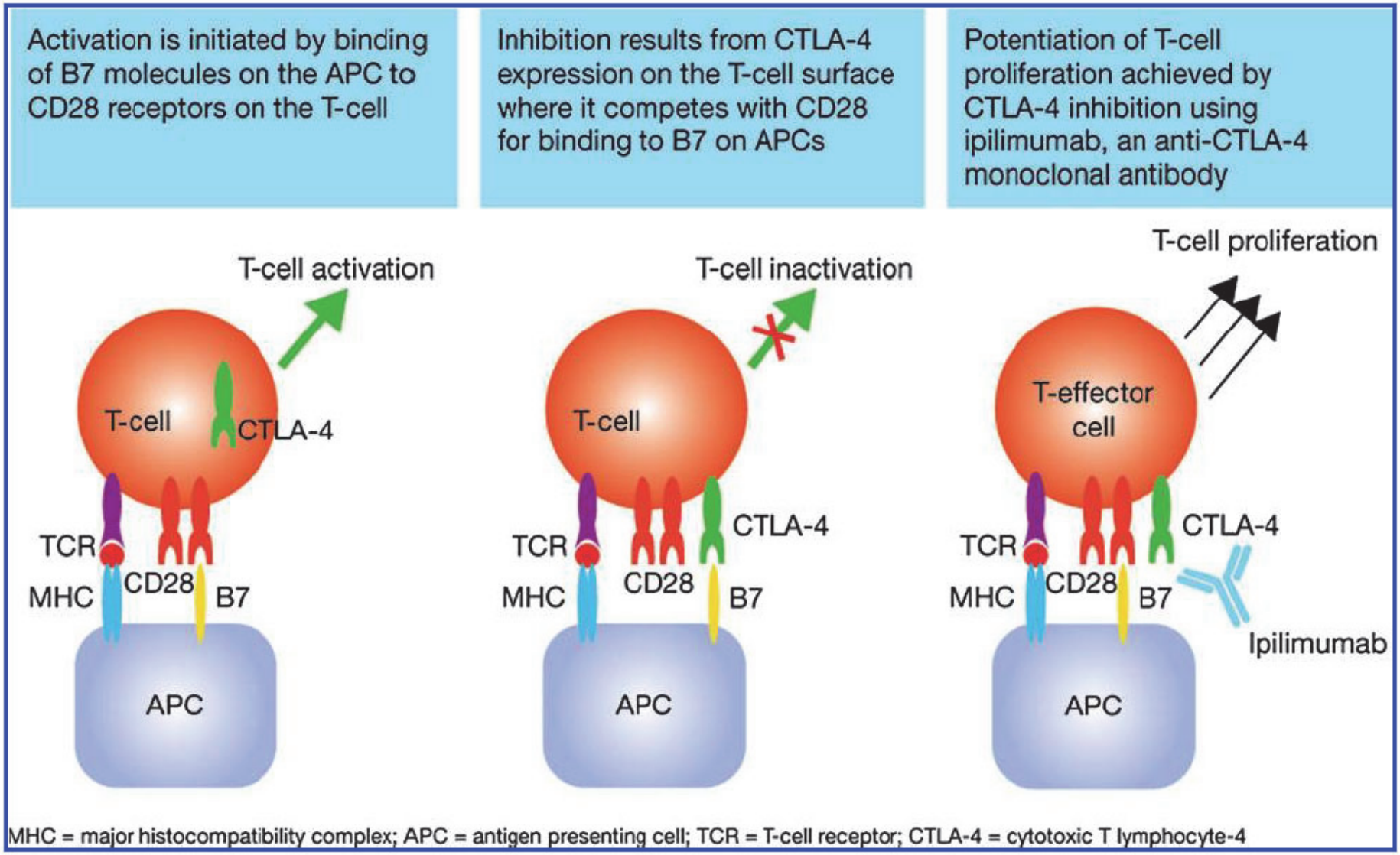


Parker, Lancet, 373:759, 2009


1 TUMOR CELLS PRODUCE FACTORS THAT STIMULATE OSTEOBLASTS TO SECRETE RANK LIGAND
2 OSTEOBLASTS AND OTHER BONE CELLS INCREASE PRODUCTION OF RANK LIGAND
RANK LIGAND
OSTEOCLAST
OSTEOBLASTS
TUMOR
4 BONE RESORPTION RELEASES GROWTH FACTORS FROM THE BONE MATRIX THAT MAY PERPETUATE TUMOR ACTIVITY
3 OVERPRODUCTION OF RANK LIGAND DRIVES INCREASED FORMATION, FUNCTION, AND SURVIVAL OF OSTEOCLASTS, LEADING TO EXCESSIVE BONE RESORPTION

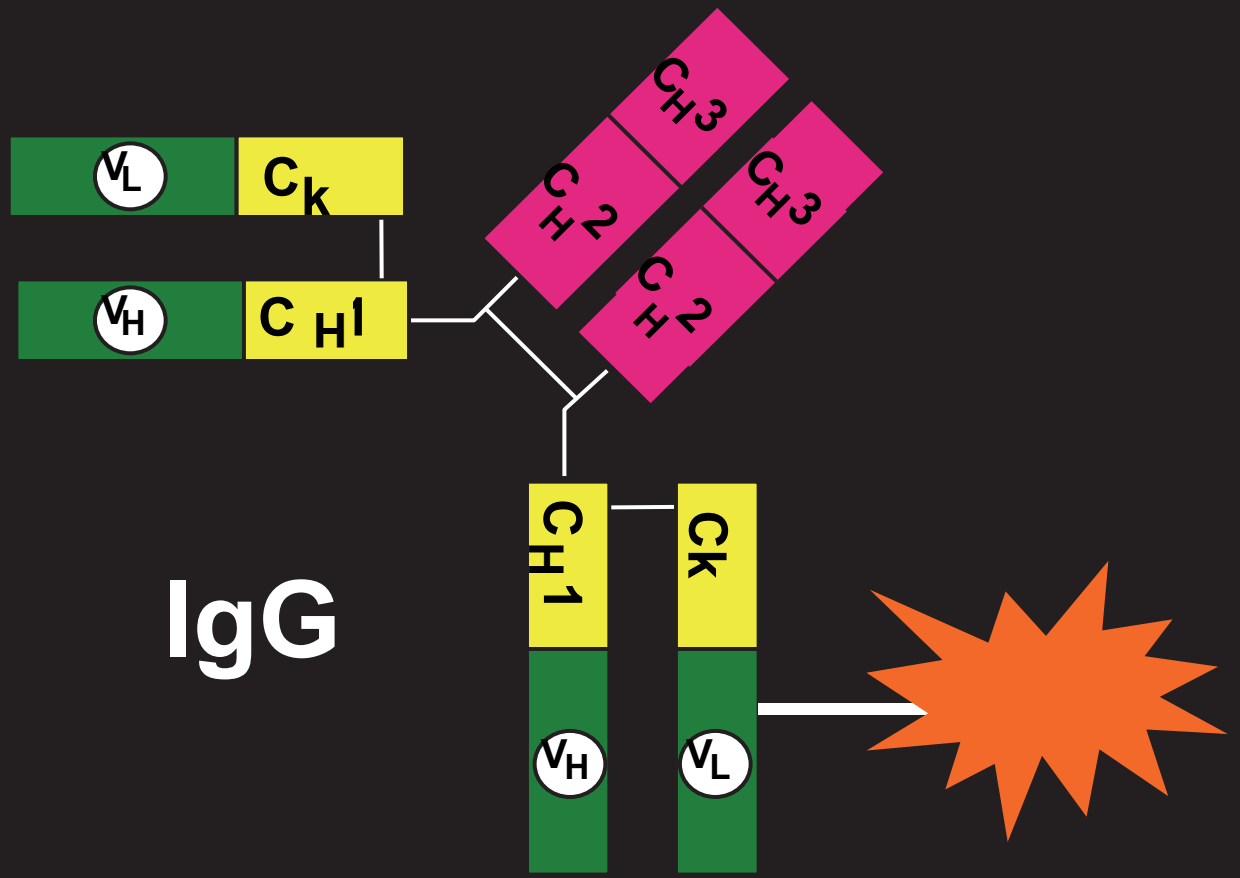
Mechanism of action of Bevacizumab

Mechanism of action of Ipilimumab

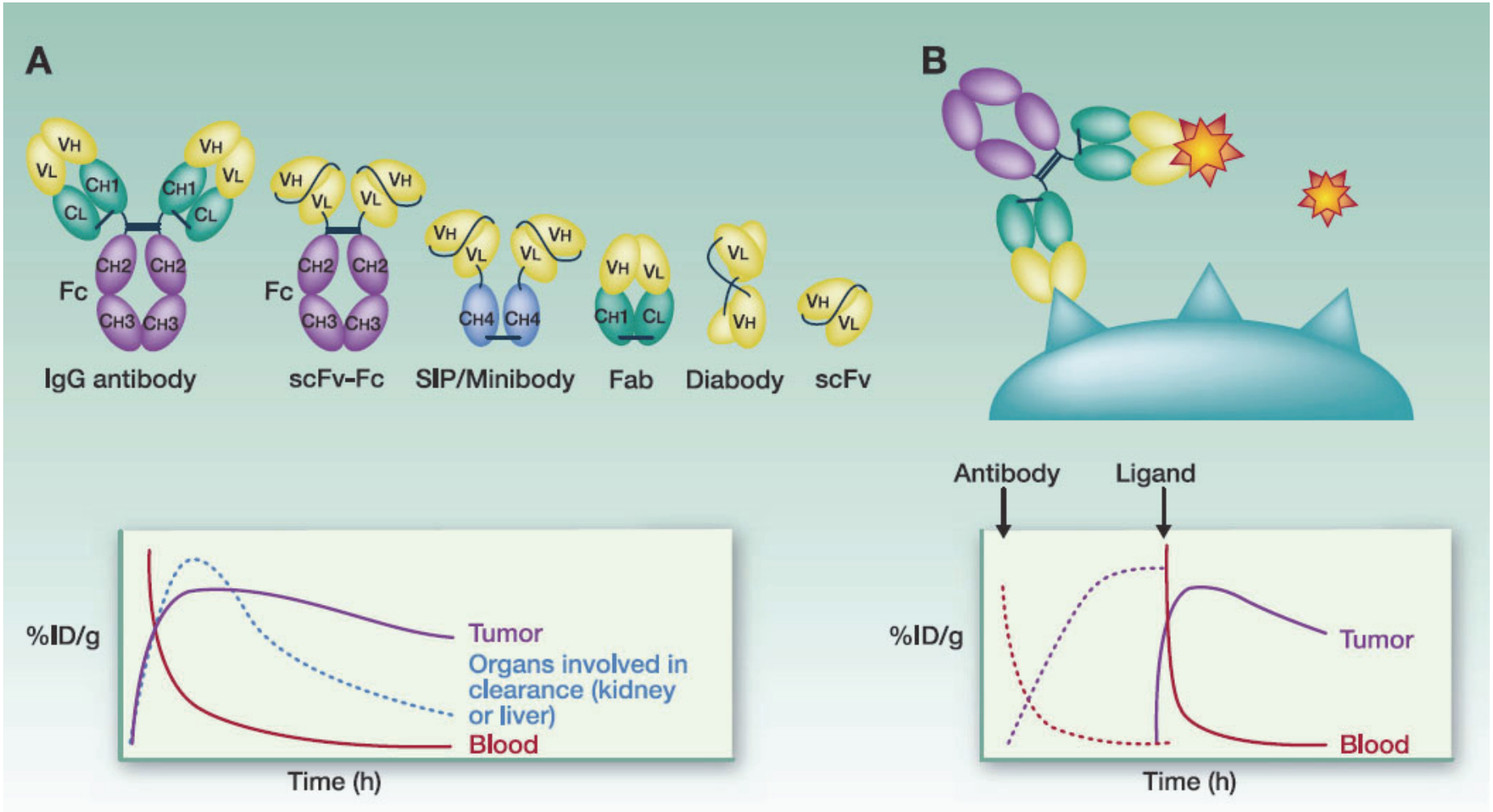


Tarhini et al., Cancer Biother Radiopharm, 25:601, 2010

Radiolabeled Monoclonal antibodies



Radioimmunotherapy



Approved (both anti-CD20)

^{90}Y-Ibritumomab tiuxetan (Zevalin)

^{131}I-Tositumomab (Bexxar)

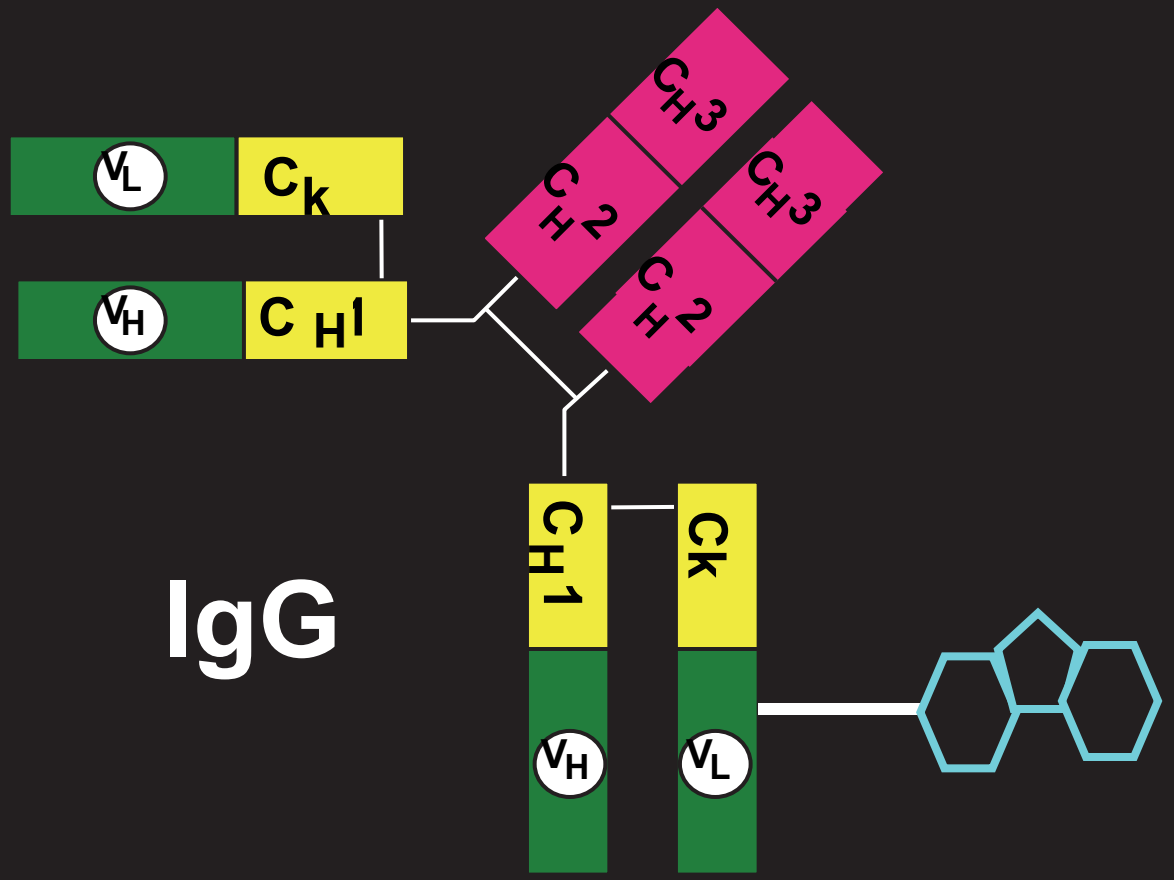
Steiner and Neri, CCR,17:6406, 2011

Radioimmunotherapy in development

^{131}I-chTNT-1/B	Tumor necrosis therapy	Brain tumors, solid tumors
^{131}I-BC8	Anti-CD45	Acute myeloid leukemia
^{177}Lu-J591	Anti-PSMA external domain	Prostate cancer
^{131}I-Metuximab	Anti-HAb18G/CD147	Hepatocellular cancer
^{177}Lu-DOTA-cG250	Anti-G250 antigen	Renal cancer
^{131}I-3F8	Anti-GD2 ganglioside	Medullo/neuroblastoma
^{131}I-L19	Fibronectin ED-B domain	NSCLC, heme tumors
^{131}I-F16	Tenascin-C A1 domain	Heme & solid tumors
^{90}Y-LL2	Anti-CD22	FL, NHL, ALL
^{90}Y-biotin/BC4-avidin	(Pretargeted)	Glioma, NHL
^{90}Y-hPAM4	anti-MUC1	Pancreatic cancer

Steiner and Neri, CCR,17:6406, 2011

Chemotherapy Immunoconjugates



Targeted Chemotherapy

Drug resistance Toxicity

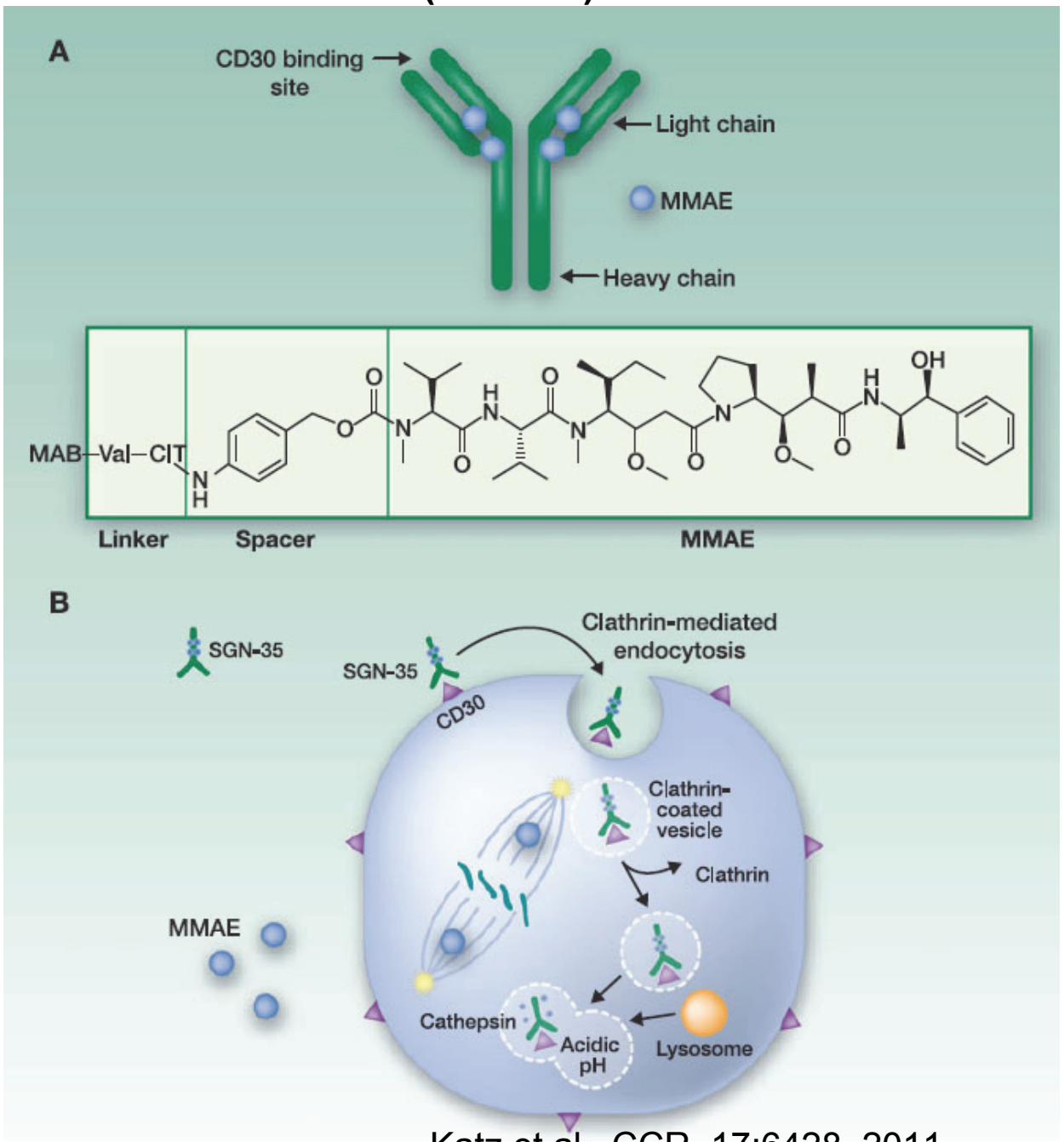
Brentuximab Vedotin (SGN-35)

Hodgkin's lymphoma
75% ORR (34% CR)

Anaplastic large cell lymphoma (ALCL)
87% ORR (57% CR)

Toxicity
Fatigue (36%)
Fever (33%)
Diarrhea, nausea (22%)
Neutropenia (22%)
Neuropathy (22%)

PML (progressive multifocal leukoencephalopathy, 2 cases)



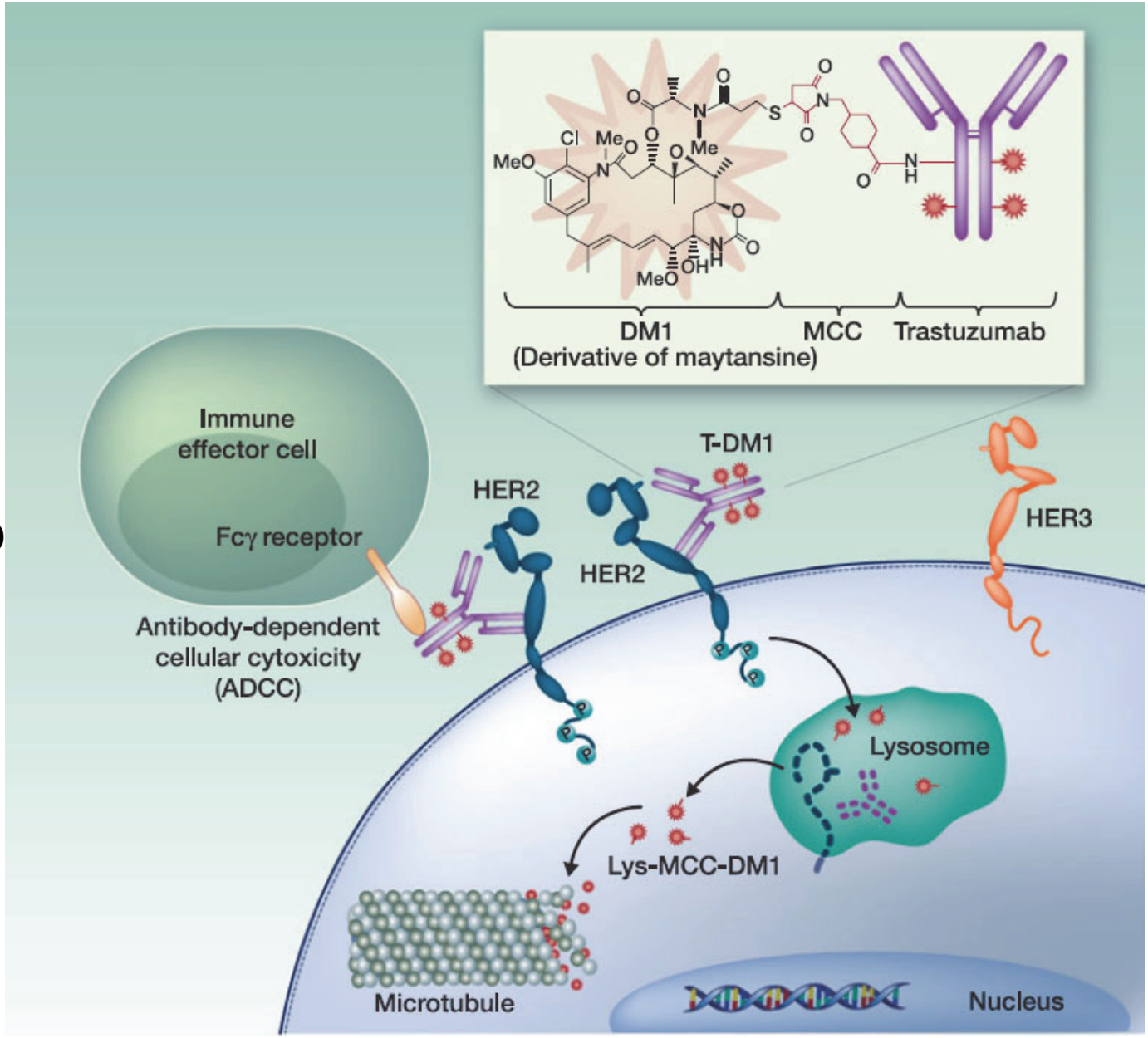
Katz et al., CCR, 17:6428, 2011

Trastuzumab Ematansine (T-DM1) for Her2+ breast cancer

- 2 phase II trials
- 100 and 112 patients
- Prior chemo + anti-Her2 therapy
- ORR 26-35%

Randomized trial

- Recurrent, advanced, Metastatic,
- Arms: T-DM1 vs T + D (D=docetaxel)
- ORR 48 vs 41%
- CR = 5 vs 1%
- PFS data pending



LoRusso, CCR, 17:6437, 2011

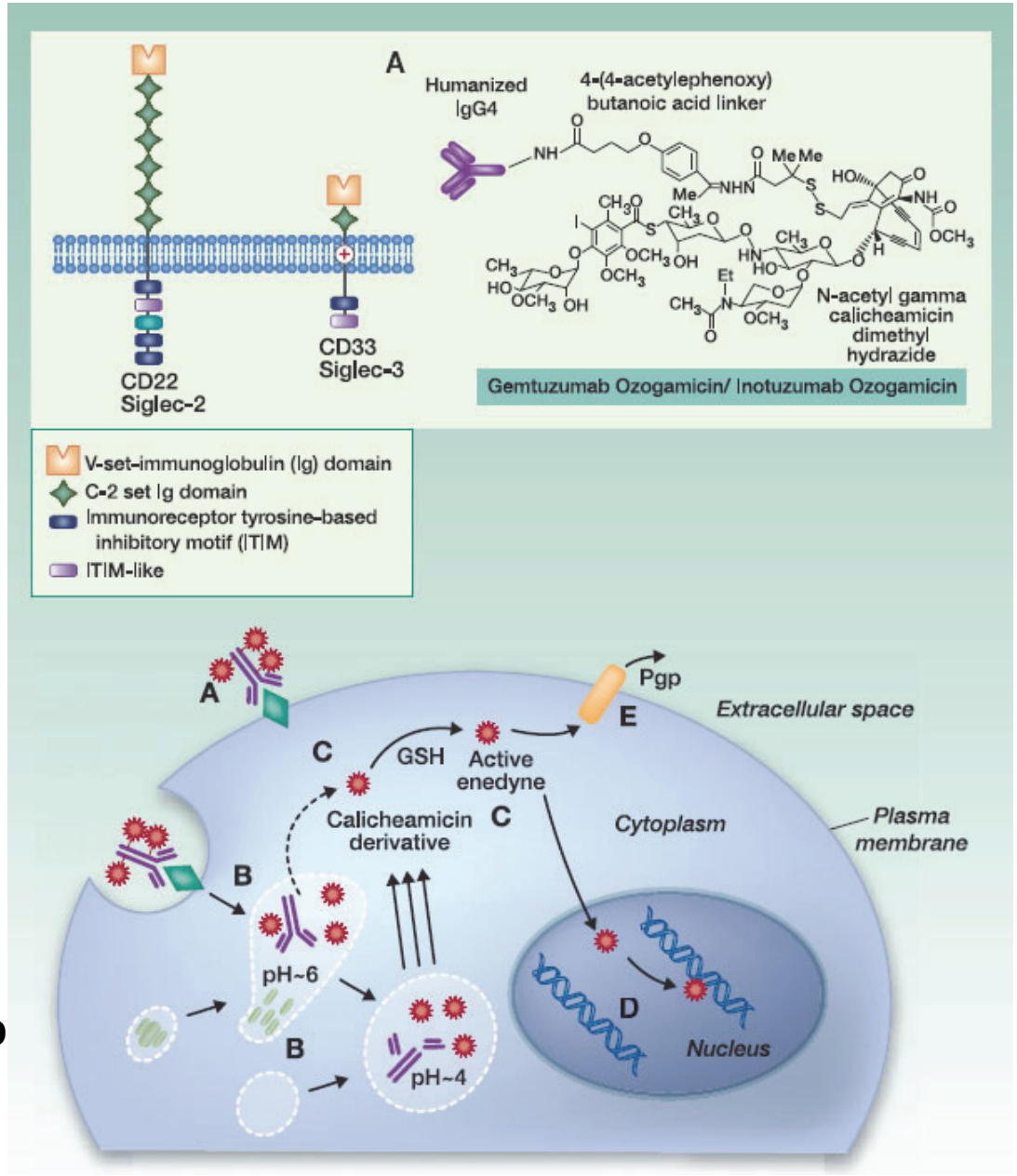
Calicheamicin targeted to CD33 and CD22

Gemtuzumab Ozogamicin (CD33)

- Approved in 2000 for CD33+ AML
- Single-agent CR rates 10-27%
- Indicated for older AML patients
- Myelosuppression #1 toxicity
- Bilirubin (23%), ALT/AST (17%)
- Veno-occlusive disease (VOD)
- Addition Daunorubicin + ARAC failed to increase CRs or OS
- Withdrawn 2010, still in Japan
- Useful for APL

Inotuzumab Ozogamicin (CD22)

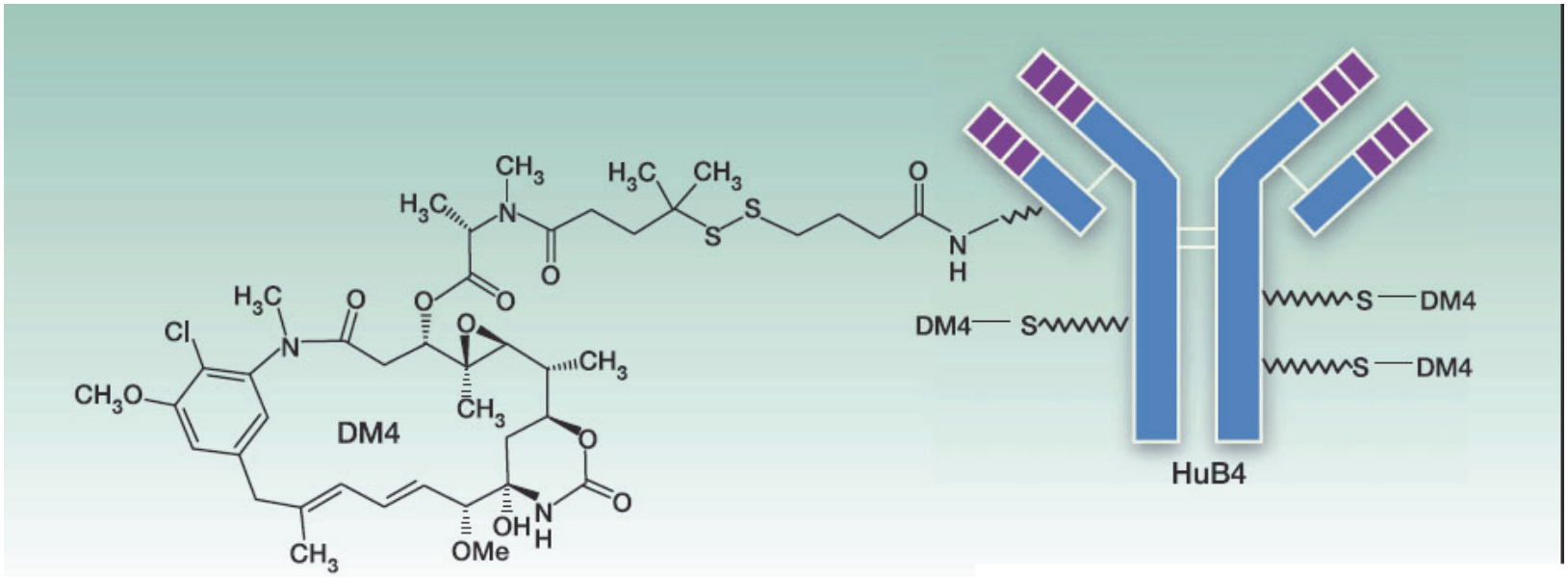
- CMC-544 contains G544
- 8 of 10 responses in NHL
- ORR 57% CR 18% in 49 ALL
- Toxicity: Fever, hypotension,
- Bilirubin, ALT/AST
- In randomized testing w/rituximab vs BenR or GemR



Richart, CCR, 17:6417, 2011

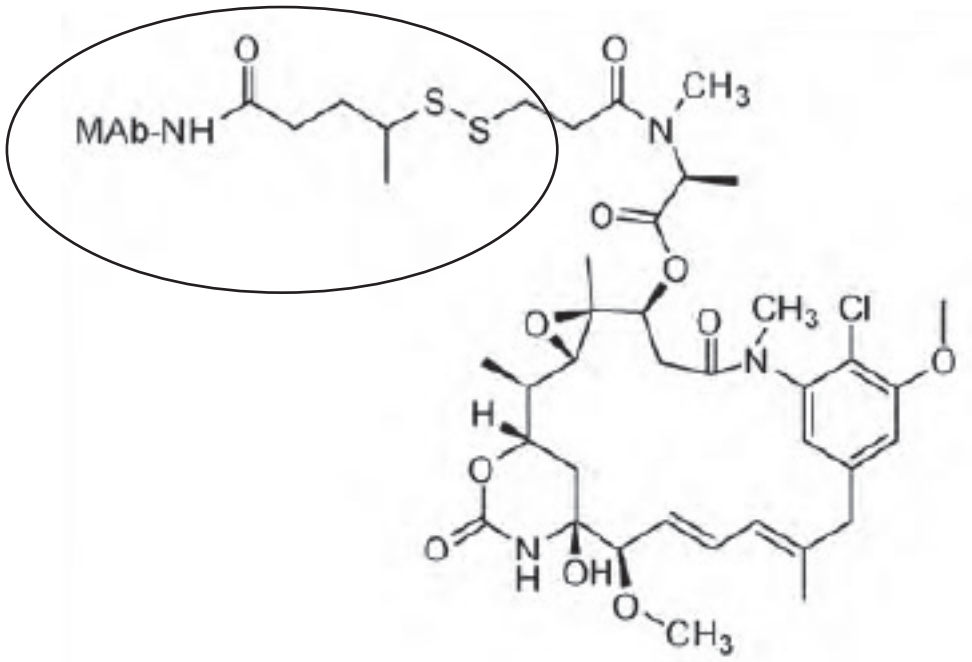
Other DM1 and DM4 Conjugates in Development

SAR3419	**anti-CD19 + DM4**	**ALL, CLL, NHL, MM (ORR 17%, n=35)**
AVE9633	**anti-CD33 + DM4**	**AML, CML, MDS**
IMGN901	**anti-CD56 + DM1**	**MM, NK-, T-cell leuk, NHL (ORR 7%, n=28)**
BT062	**anti-CD138 + DM4**	**MM**

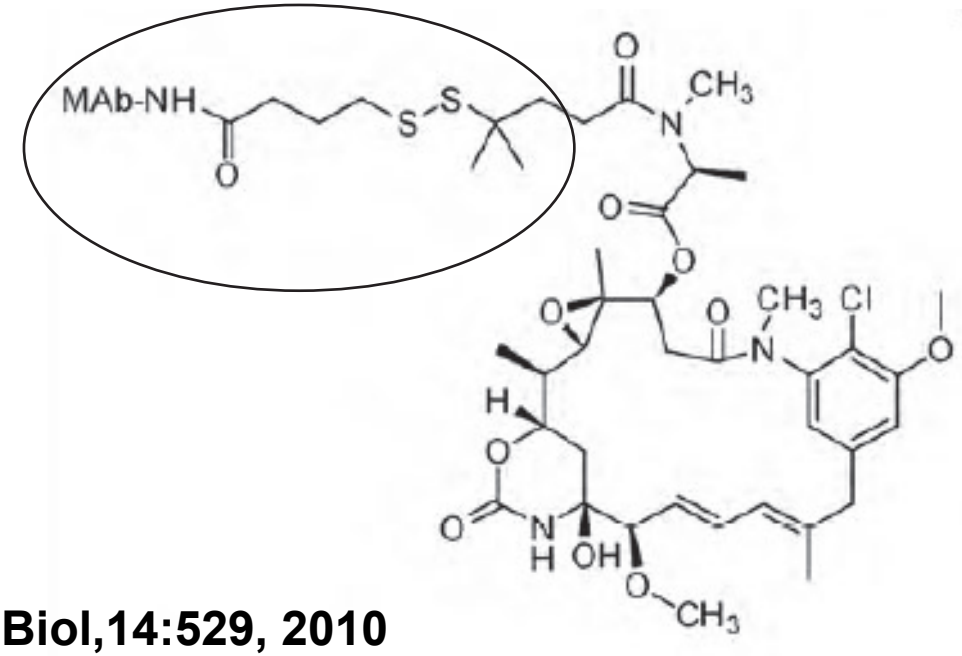


Blanc et al., CCR, 17:6448, 2011 and FitzGerald et al., Cancer Res, 17:6300, 2011

DM1

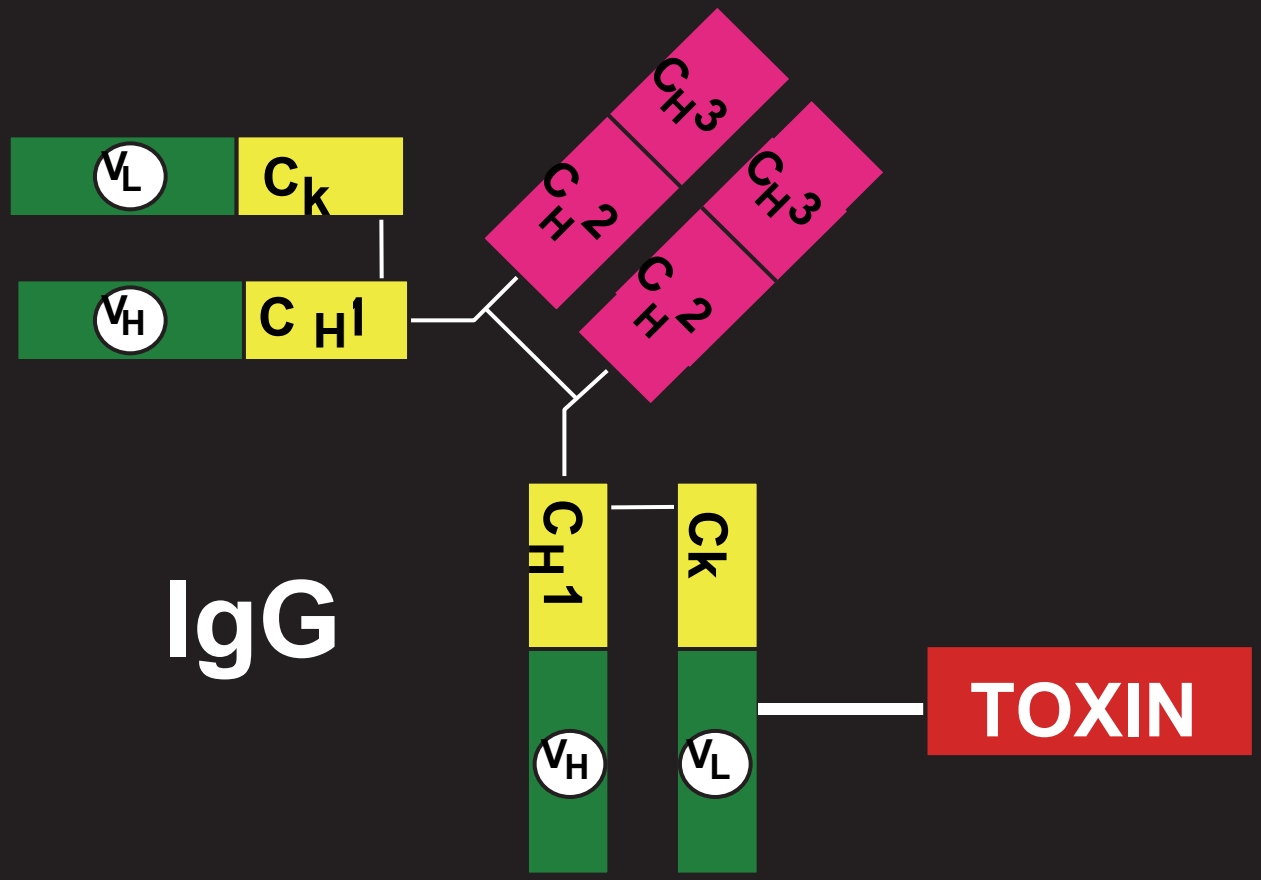


DM4



Alley et al., Curr Opin Chem Biol,14:529, 2010

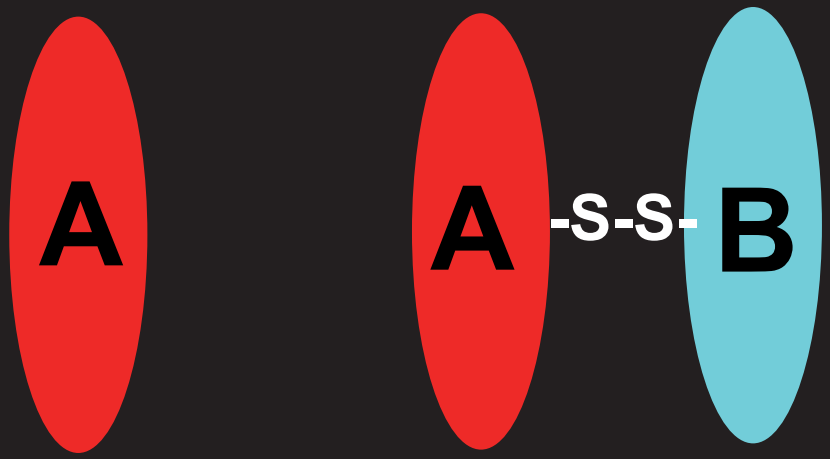
Immunotoxins



Inhibition of protein synthesis
Apoptosis

Immunogenicity

PROTEIN TOXINS FROM PLANTS

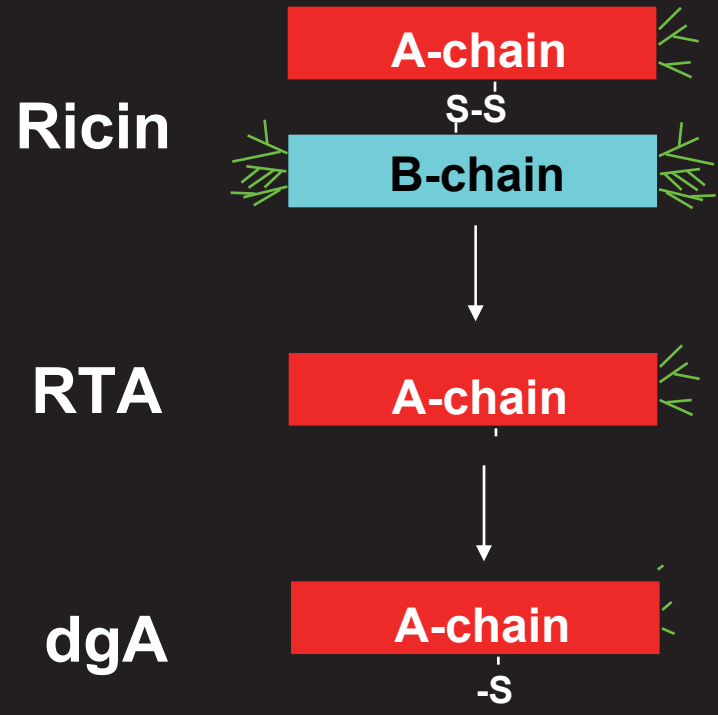


Hemitoxins:

Saporin
PAP
Gelonin
BRIP
Momoridin
Trichosanthin

Holotoxins:

Ricin
Abrin
Mistletoe
Lectin
Modeccin
Viscumin

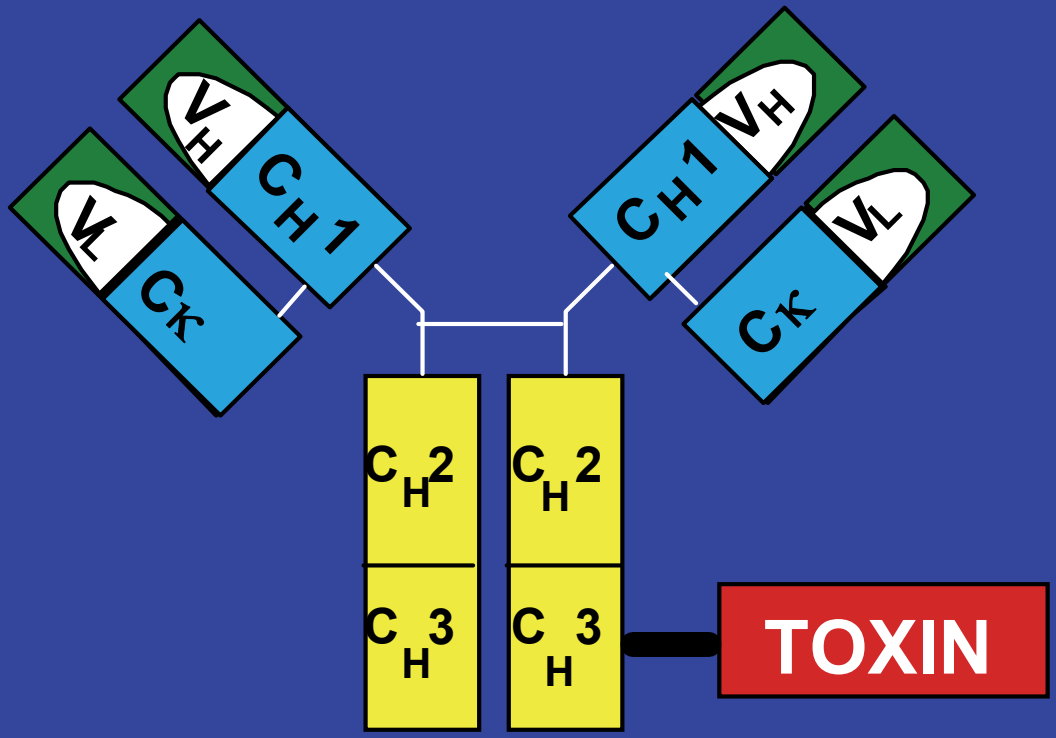


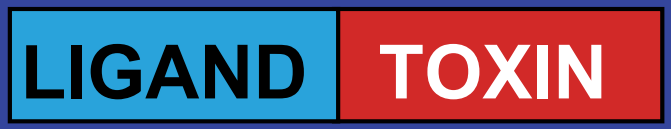
- Recent examples:
- Anti-CD19-dgA and anti-CD22-dgA in ALL (18% CR)

Herrera et al., J Ped Hemat Oncol, 31:936, 2009

- rGel fused to:
  - Ligands targeting Her2
  - BLyS
  - $VEGF_{121}$

Lyu et al., Meth Enzymol, 502:167,2012



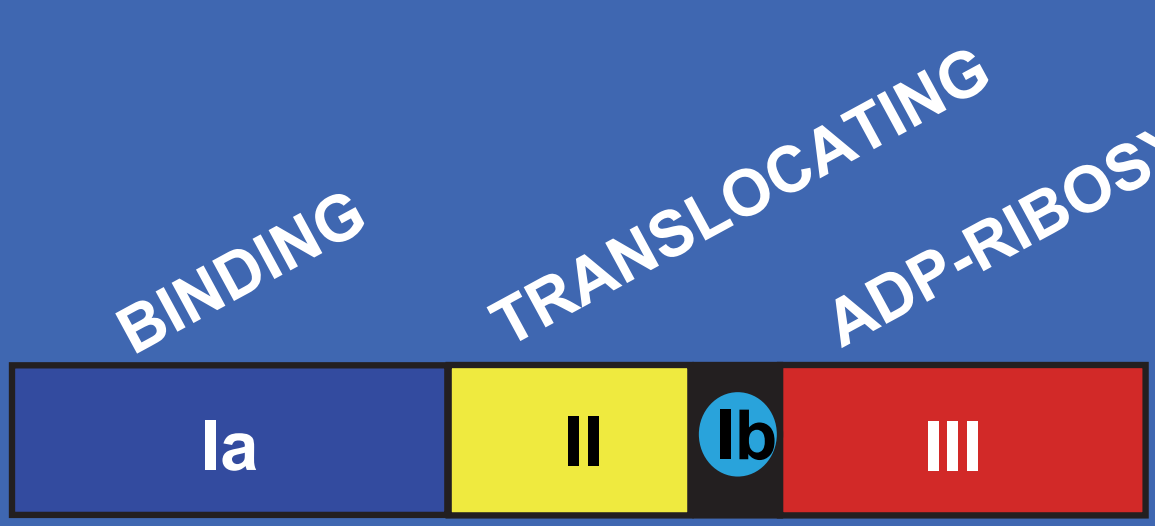


CHEMICAL CONJUGATE

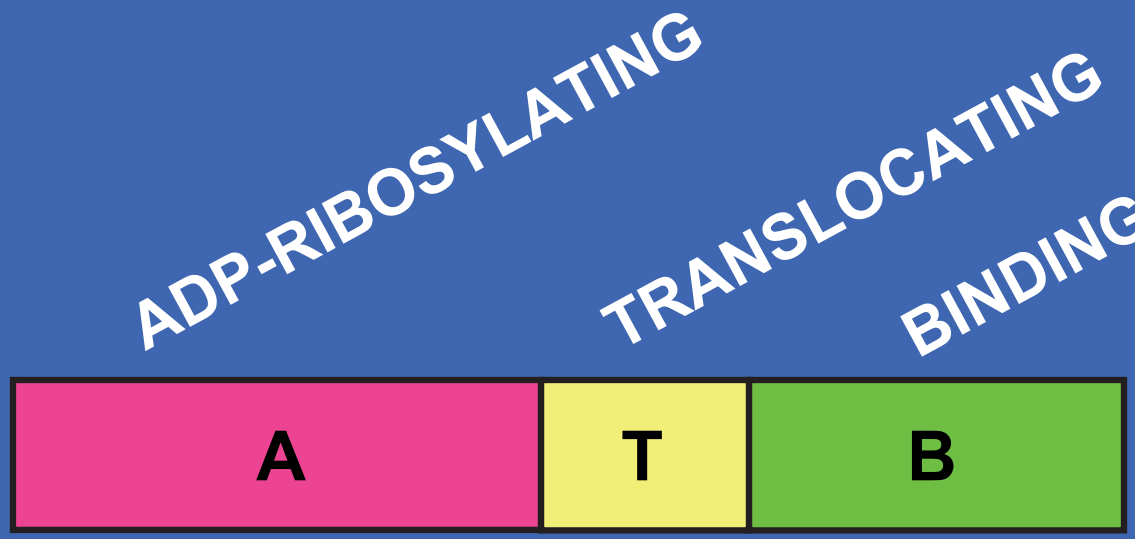
FUSION TOXIN

Advantages of fusion toxins:

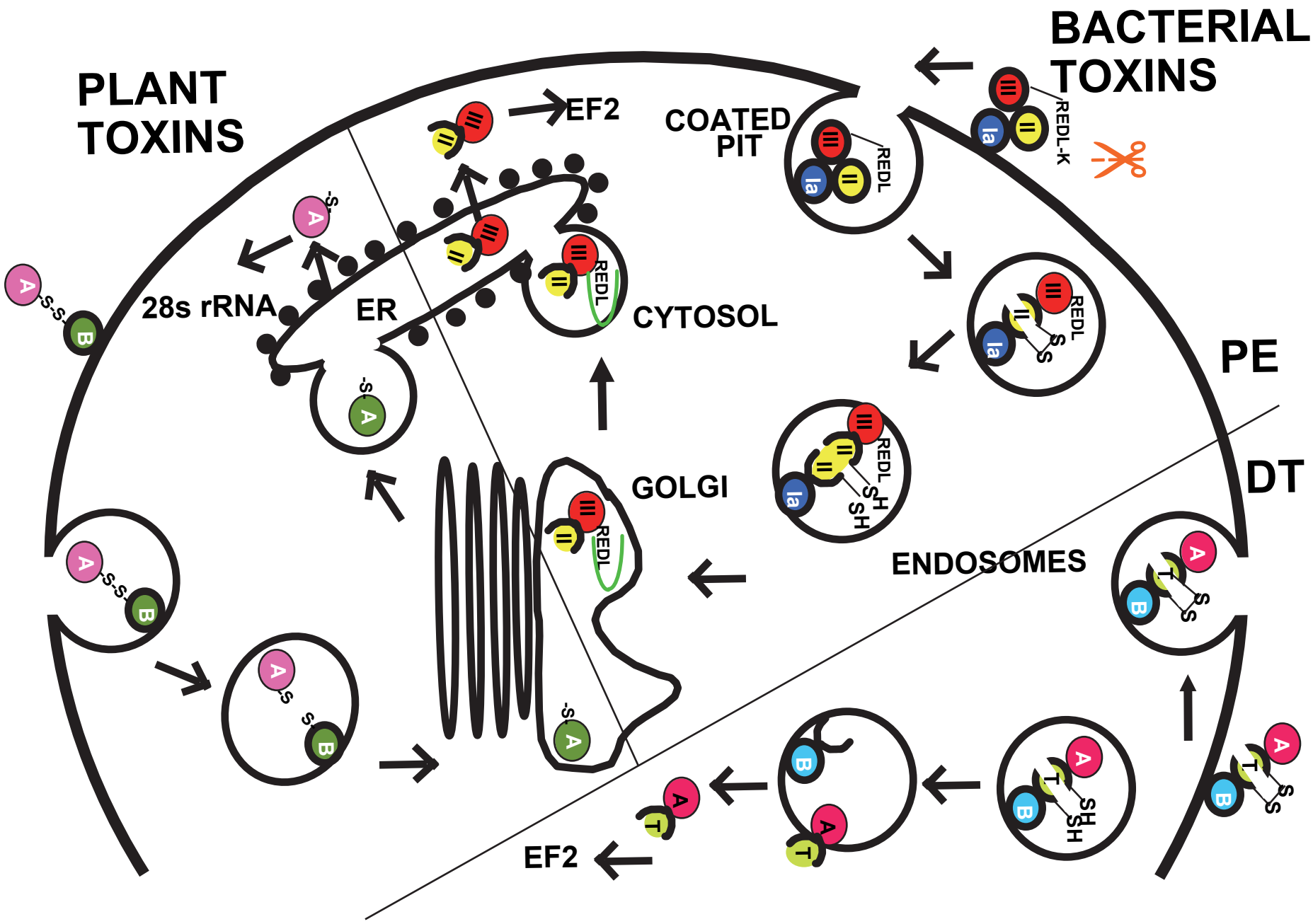
1. More Homogenous
2. Easier to Produce
3. Smaller size

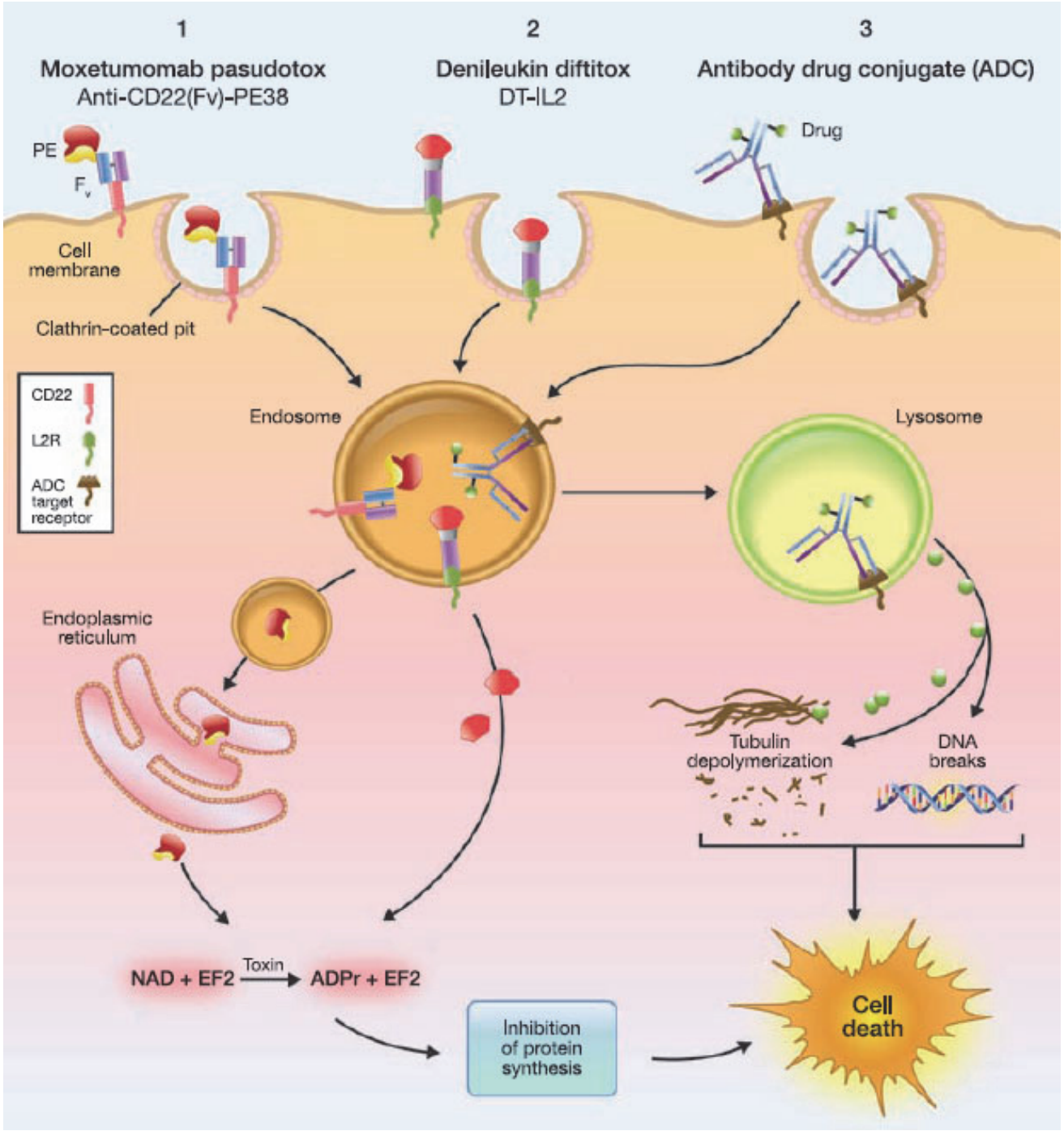


PSEUDOMONAS EXOTOXIN



DIPHTHERIA TOXIN


PLANT TOXINS
BACTERIAL TOXINS
EF2
COATED PIT
REDL
REDL-K
28s rRNA
ER
CYTOSOL
PE
DT
GOLGI
ENDOSOMES
EF2


1
Moxetumomab pasudotox
Anti-CD22(Fv)-PE38
2
Denileukin diftitox
DT-IL2
3
Antibody drug conjugate (ADC)
PE
F_v
Drug
Cell membrane
Clathrin-coated pit
CD22
L2R
ADC target receptor
Endosome
Lysosome
Endoplasmic reticulum
Tubulin depolymerization
DNA breaks
NAD + EF2
Toxin
ADPr + EF2
Inhibition of protein synthesis
Cell death

SUMMARY OF PURIFICATION

Fermentation (*E. coli* BL21/λDE3)

↓ IPTG induction

E. coli cell paste

↓ Triton X-100 washes

Inclusion bodies

↓ Guanidine-DTE

Denatured-reduced recombinant protein

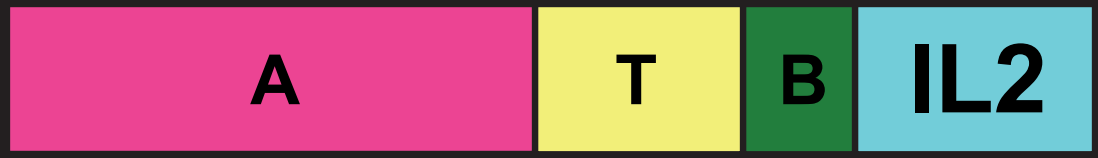
↓ 100x dilution into refolding buffer

Refolded protein

↓ Anion exchange sizing chromatography

Purified recombinant immunotoxin

Denileukin diftitox
$DAB_{389}IL2$ (Ontak)



Phase I: 5 CRs, 8 PRs In 35 with CTCL, MTD 27ug/Kg qd x5

Phase III: 10% CRs, 30% ORR IN 71 With CTCL, @9-18 ug/Kg/d x5

Phase III: Placebo vs 9 vs 18 ug/Kg/d x5

ORR 16% vs 38% vs 49%

CR 2% vs 11% vs 9%

PFS 124d vs 794d vs >971d

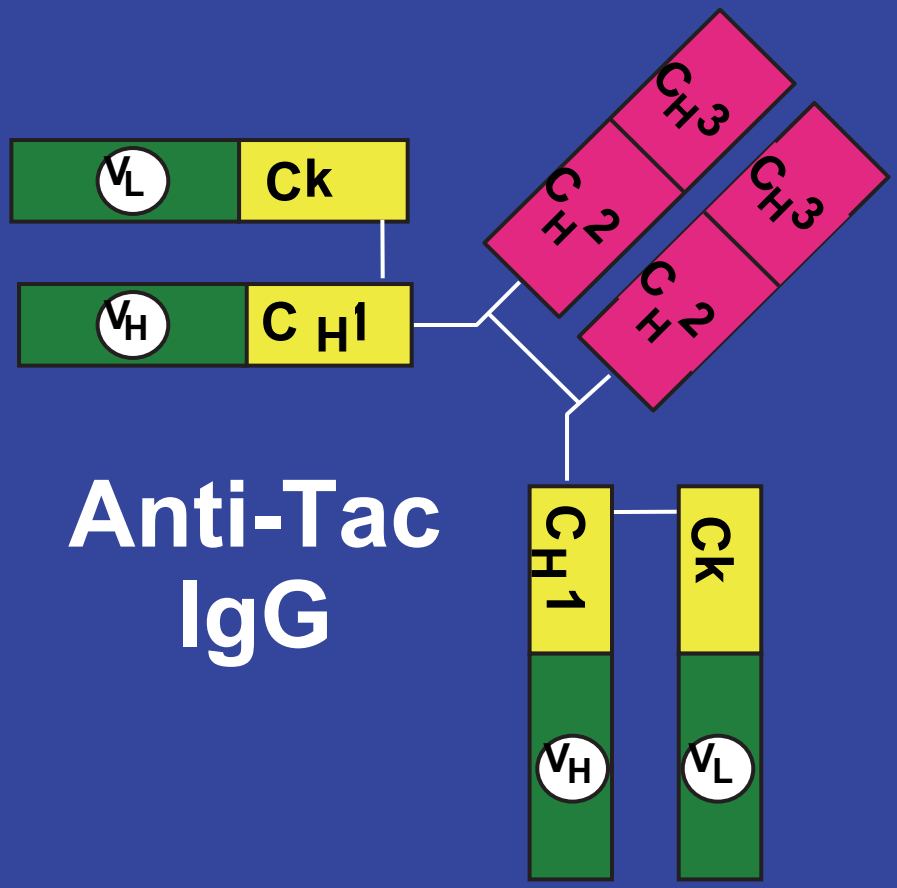
Only recombinant toxin approved by FDA

Toxicity: Fatigue, CLS, hypersensitivity (use steroids)

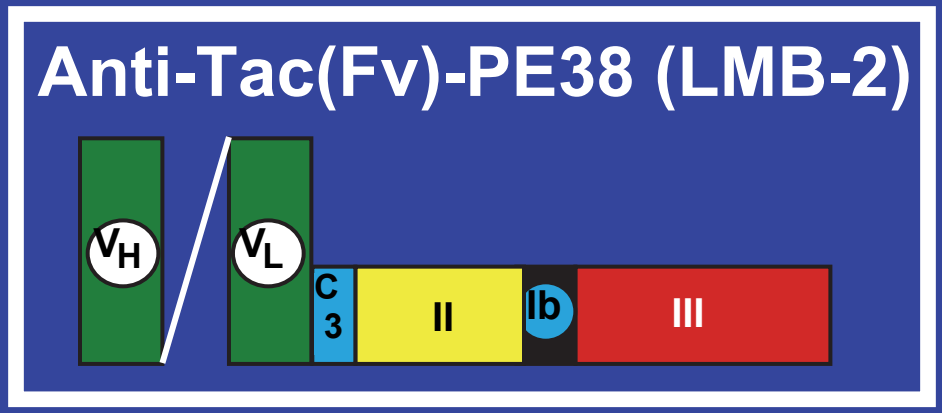
Other DXs: PTCL, psoriasis, CLL (4/18 pr), NHL (7% cr,18%pr)

Prince et al., JCO, 28:1870, 2010, Lansigan et al., Cancer Manag Res 2:53, 2010.

Recombinant Immunotoxins

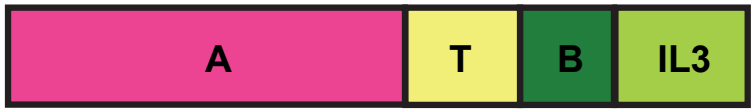


Anti-Tac IgG

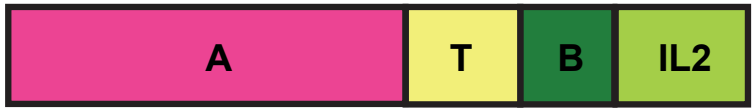


PE

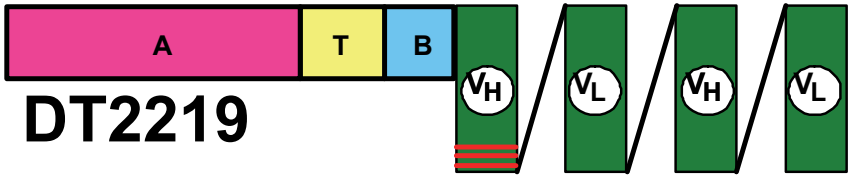
DT388-GM-CSF (DTGM)



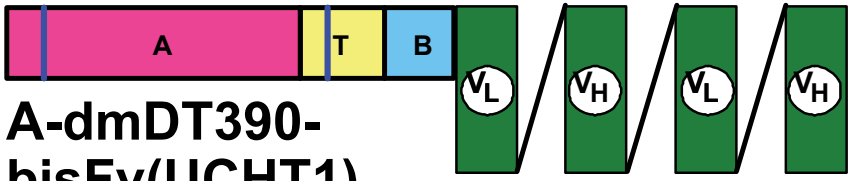
DT388-IL3 (DTIL3)



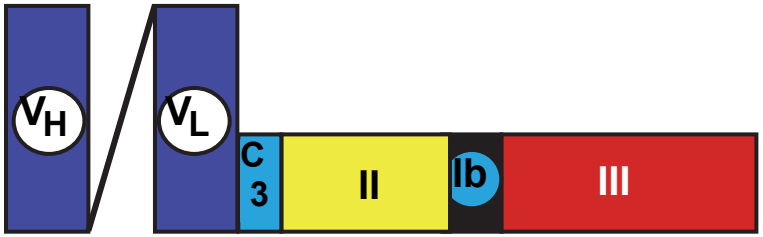
DAB389IL2 (Ontak)
(denileukin diftitox)



DT2219

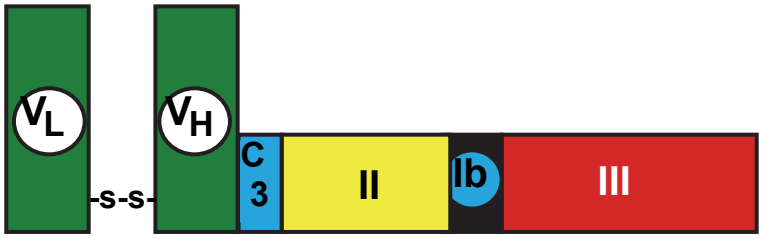


A-dmDT390-
bisFv(UCHT1)

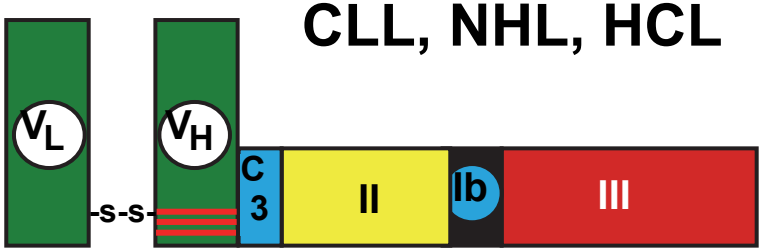


Anti-Tac(Fv)-PE38 (LMB-2) (CD25)

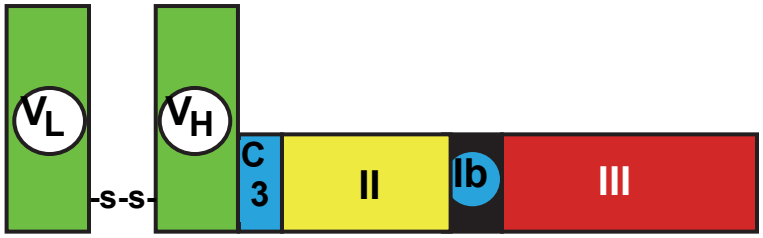
IL4(38-37)-PE38KDEL (GBM)



RFB4(dsFv)-PE38 (BL22)
CLL, NHL, HCL

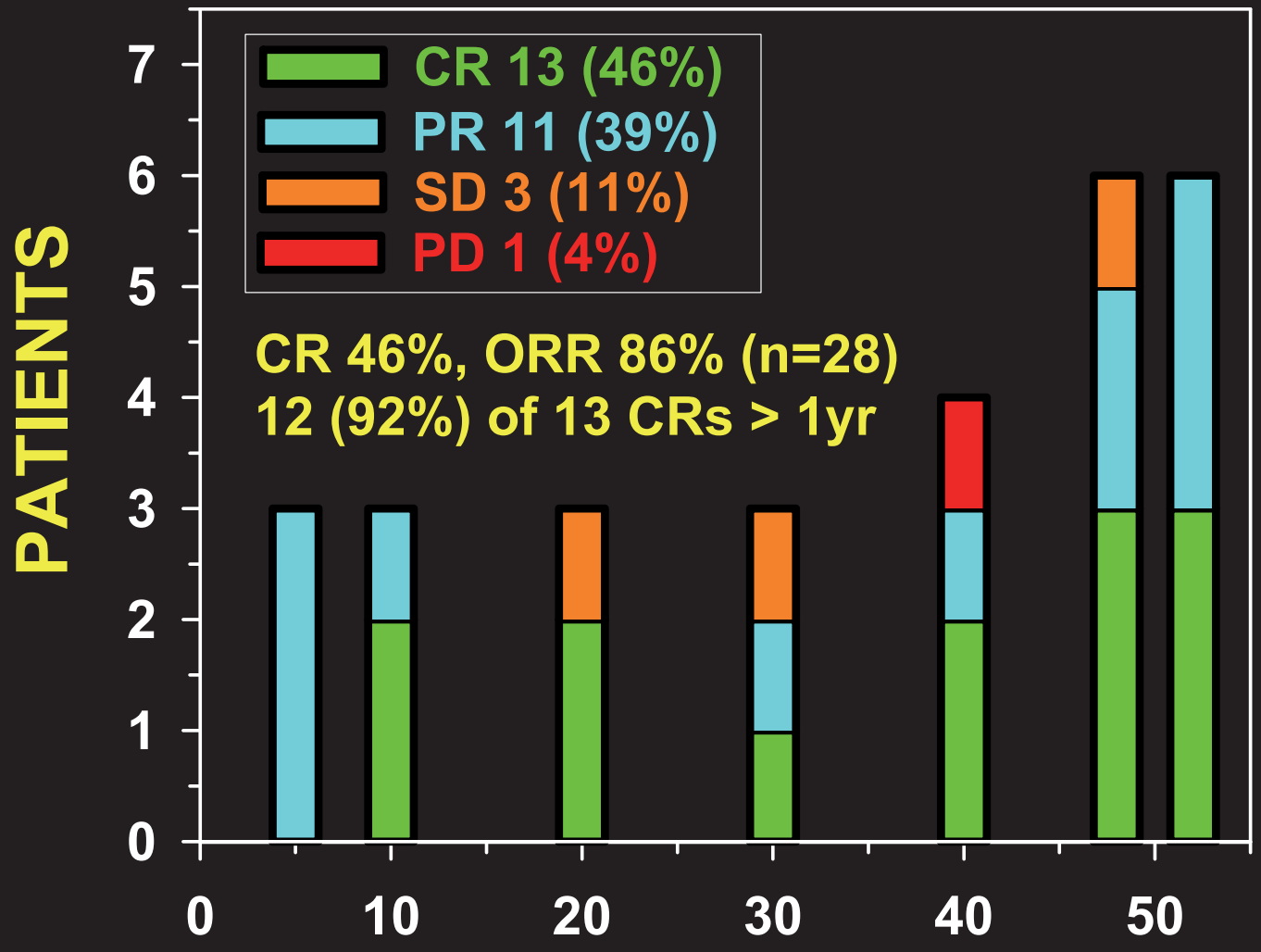


BL22VH 100-100b SSY->THW
Moxetumomab pasudotox

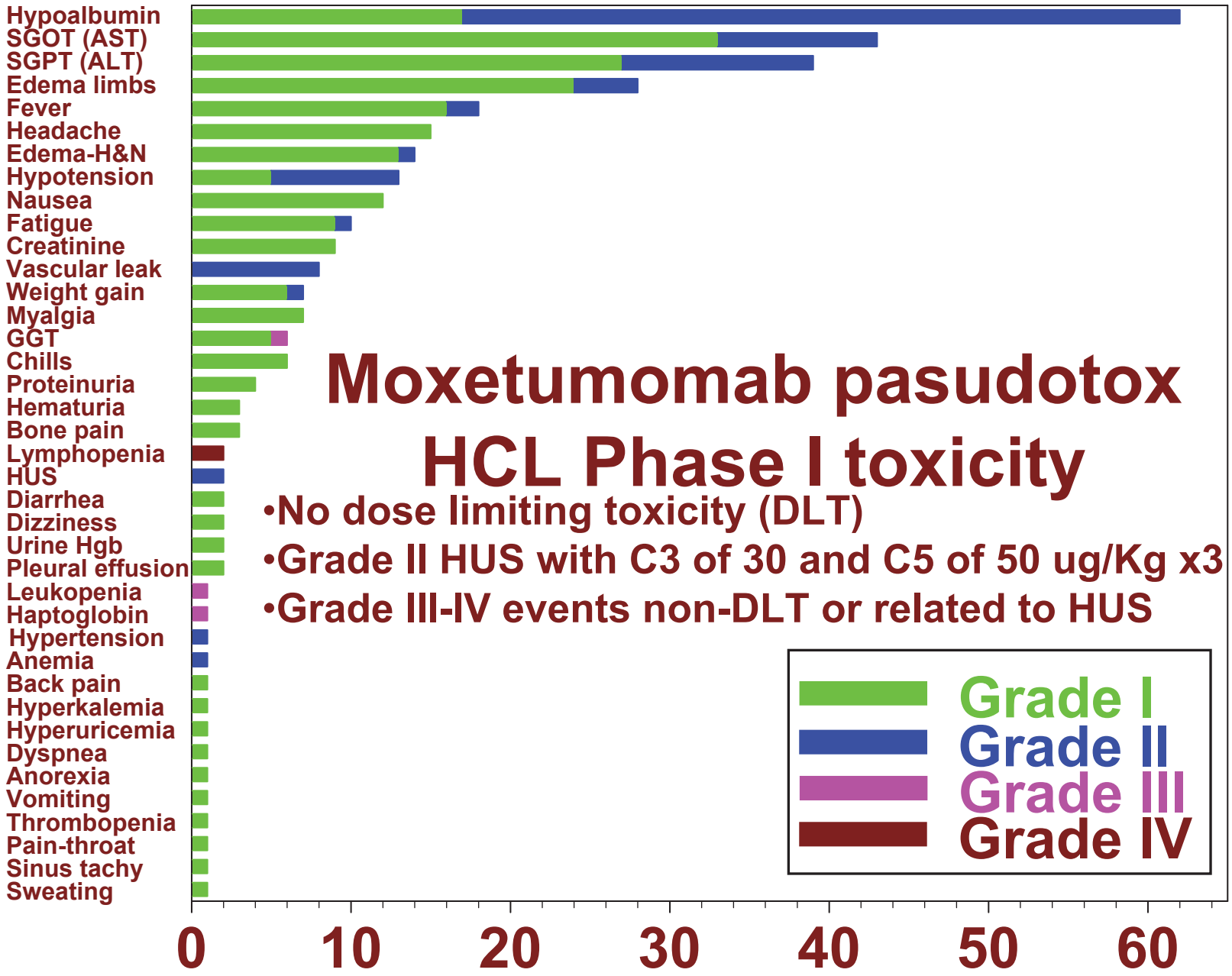


SS1(dsFv)-PE38 (SS1P)
Mesothelioma

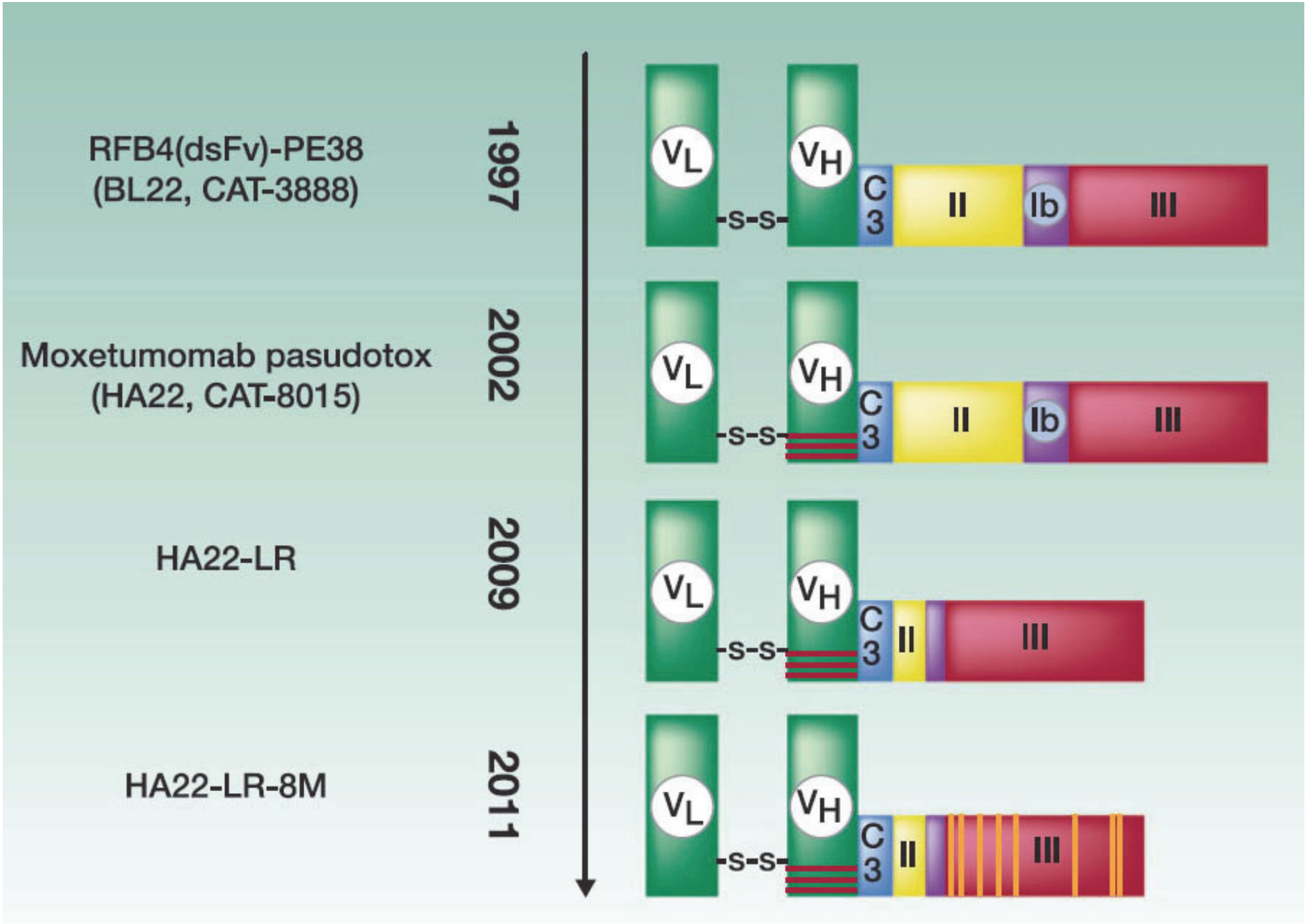
HCL Response to HA22
(CAT-8015 or moxetumomab pasudotox)



Kreitman et al., JCO, 2012



Kreitman et al., JCO, 2012.

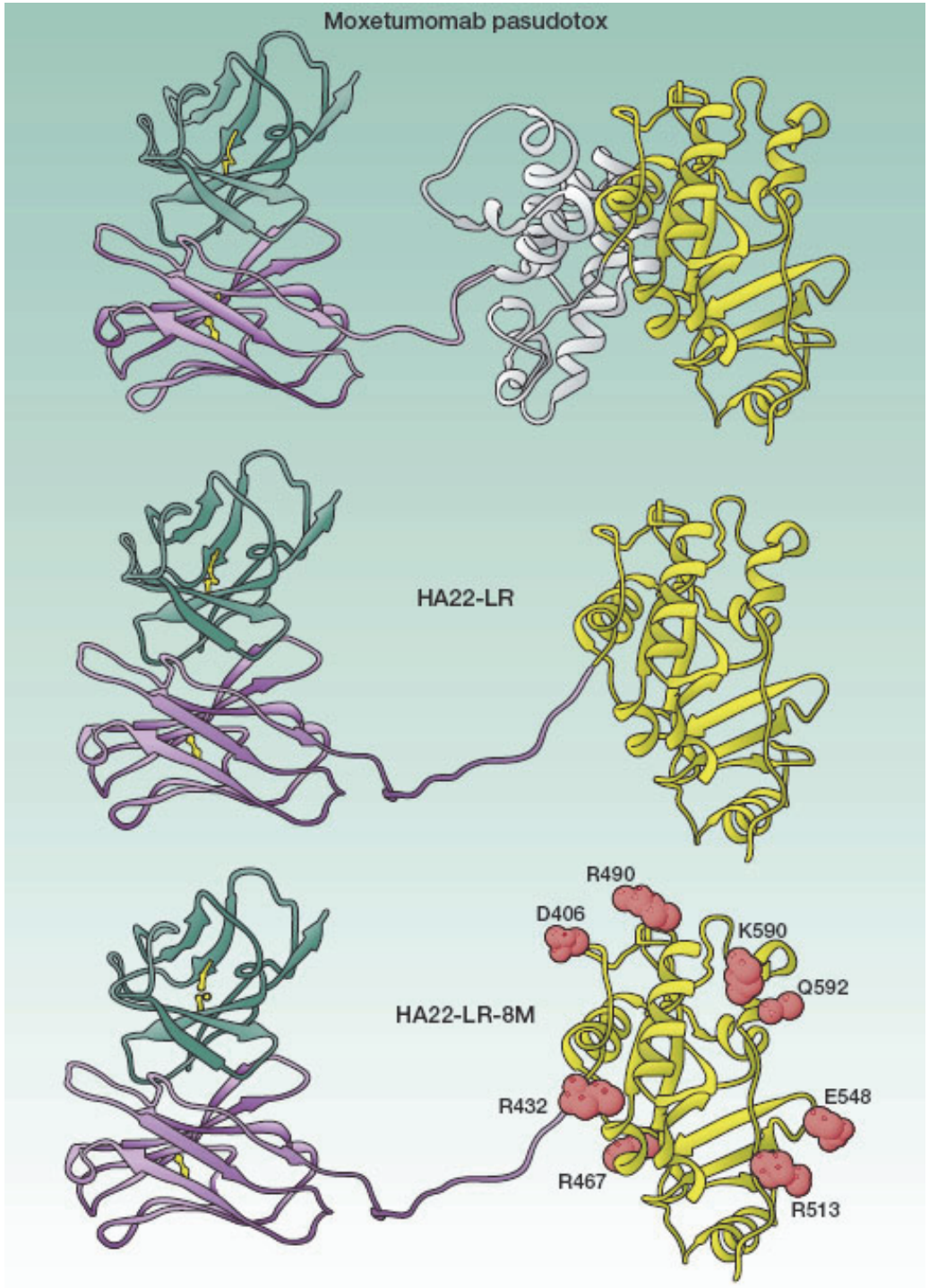


Kreitman et al, CCR, 17:6398, 2011

Moxetumomab pasudotox: Median 8-fold more cytotoxic than BL22

HA22-LR: Median 16-fold more cytotoxic than moxetumomab pasudotox

HA22-LR-8M: Activity similar to HA22-LR



Kreitman et al, CCR, 17:6398, 2011

Conclusions

- The most common Mab-based therapies for cancer are unlabeled MAbs, but the 3 classic mechanisms of action, 1) apoptosis, 2) CDC and 3) ADCC only work for a minority of targets.
- Radioimmunotherapy is effective in CD20+ NHL (early and relapsed), but its use and development of new agents have been limited.
- Antibody-drug conjugates have used the high cytotoxicity of auristatins, maytansenoids, and calicheamicin to produced several clinically useful conjugates for both solid and hematologic tumors.
- Targeted protein toxins exploit the most powerful killing agents known, able to kill cells catalytically with as few as 1 molecule. Efficacy is limited by immunogenicity particularly in solid tumors.
- Denileukin diftitox is a targeted toxin fusion protein approved for CTCL, with some efficacy demonstrated in PTCL, CLL and NHL.
- Recombinant immunotoxins have demonstrated clinical efficacy, particularly moxetumomab pasudotox for HCL and SS1P for mesothelioma. Immunogenicity may be reduced by humanizing the toxin and by combination with chemotherapy.