

Lecture 9: Antibodies & Antibody Conjugates - targeted cell killing agents

Monoclonal antibodies can be identified quickly by the 'mab' at the end of their name. They can be "naked" or "conjugated" depending on whether they are attached to something else. Furthermore, if the antibody is of mouse origin they end with 'momab'. If the mab is part human and part mouse, it is called "chimeric". If it is purely human, it is called humanized and the name often ends in 'umab'.

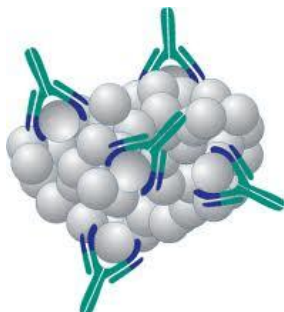
Introduction of foreign molecules (e.g. proteins, antibodies, bacteria, viruses, nucleic acids) can cause immune reactions in the host. Administration of antibodies of mouse origin can result in the human anti-murine antibody (HAMA) response. The HAMA response can range from mild to severe and it has been estimated that this occurs in 30-50% of patients. The development of chimeric and especially humanized antibodies can help reduce this type of immune response.

Monoclonal antibodies (Mabs) usually target antigens on cell surfaces (Figure 1). Sometimes the mabs target soluble factors (ligands) in plasma. Regardless, this immunologic targeting provides for their great selectivity. The consequent cell-antibody complex recruits endogenous immunomodulator and cell-destroying entities (macrophages and natural killer cells), which recognizes the cell-antibody complexes as foreign and destroys them.

Conjugated mabs are complexes that link antibodies with radioisotopes, or cytotoxic molecules, or anything that has cell killing power. This strategy combines the specificity of the monoclonal antibody with the extra cell killing power of the toxic payload.

The pharmacokinetics of mabs is complex and dependent on whether they bind to cell surface antigens or soluble factors. Being large proteins, the mononuclear phagocyte system, formerly called the reticuloendothelial system (RES), is important for the degradation and elimination of antibodies that bind soluble factors. Macrophages routinely remove senescent erythrocytes, leukocytes, and megakaryocytes by phagocytosis and digestion. The spleen contains a large number of macrophages, as does the liver as Kupffer cells. The lymph nodes, lungs and brain also contain macrophages. If the antibodies bind cell surface antigens, the antibodies tend to be internalized into the cells and antibody clearance is often dependent on the number of cells expressing the antigen. This can also give rise to non-linear kinetics. Mabs and conjugated mabs are always administered IV.

Figure 1: The binding of naked antibody molecules to antigen proteins on a cell surface.



1. **Naked antibodies** (nothing attached to them):

Rituximab (Rituxan); approved in 1997

Uses: Refractory B-cell non-Hodgkin's lymphoma; greater than 90% of B-cell NHL have the CD20 antigen which is not expressed on normal cells; also for CD20+ CLL (in combination with fludarabine and cyclophosphamide). CD20 is called the B-cell antigen.

Mechanism: A chimeric antibody (IgG1) that binds specifically to CD20 antigens on cell surface.

Toxicities: Potential severe immune/allergic reactions, severe mucocutaneous reactions, tumor lysis syndrome, progressive multifocal leukoencephalopathy (PML)* (all in black box warning).

ADME: Elimination t_{1/2} can range widely among patients (14-60 days).

Note(s): *Immunodeficiency or immunosuppression allows JCV (John Cunningham virus) to reactivate. In the brain, it causes destruction of oligodendrocytes (PML can be fatal). JC virus is very common in the human population (70-90% of humans have it). Agent is also useful for rheumatoid arthritis (RA) since B-cells involved in the pathogenesis of RA.

Trastuzumab (Herceptin); approved in 1998

Uses: Breast cancer but only if HER2/neu +, in combination with chemotherapy (e.g. a taxane, anthracycline, and cyclophosphamide). The over-expression of this HER2 protein occurs in nearly 30% of breast cancer patients. Also used in gastric cancer in combination with chemotherapy (e.g. cisplatin and 5-FU or capecitabine) if gastric tumors are HER2+.

Mechanism: A humanized antibody that binds specifically to the cell surface receptor HER2. This receptor is also called neu or EGFR-2.

Toxicities: Immune/allergic reactions, pulmonary edema, cardiotoxicity (all in black box warning).

ADME: Elimination t_{1/2} is 2-12 days; increases with dose.

Note(s): A genetic assay (HercepTest) exists to determine the expression level of HER2 in tumors. Testing is required prior to administration of Herceptin. If patient is HER2-, then no value using Herceptin. In fact, it can just add extra/additional toxicities. A drug conjugate with trastuzumab was approved in 2013 (called T-DM1, more on this below).

Alemtuzumab (Campath); approved in 2001.

Uses: Chronic lymphocytic leukemia (B-cell) as a single agent for previously untreated patients.

Mechanism: A humanized antibody that specifically binds to CD52 antigens on cell surface. CD52 is another antigen on lymphocytes, but more broadly than CD20. Some bone marrow cells, including some CD34⁺ cells, express variable levels of CD52.

Toxicities: Severe immune/allergic reactions, severe myelosuppression, serious infections* (all in black box warning).

Note(s): In addition to premedicating for immune reactions, important to premedicate with antibiotics to prevent infections* (e.g. trimethoprim/sulfamethoxazole for *Pneumocystis carinii* pneumonia (PCP)). Some bone marrow cells have the CD52 antigen and therefore are targeted. This leads to a specific immunosuppression.

Cetuximab (Erbix); approved in 2004; modified 2012

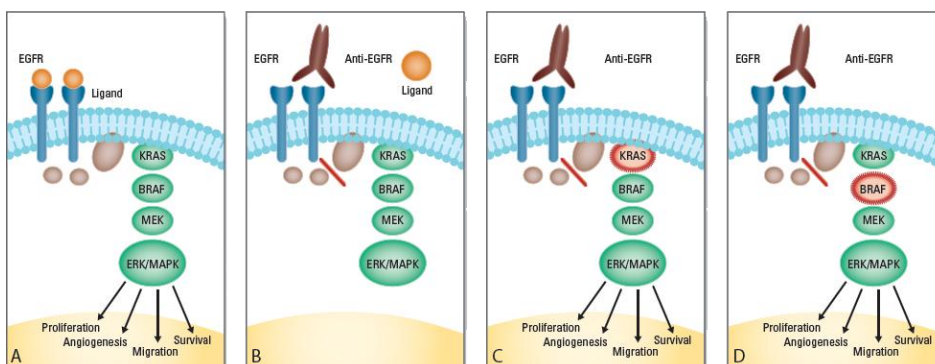
Uses: For metastatic colorectal cancer (CRC) with FOLFIRI but only for KRAS mutation negative; also for first-line treatment of locally or regionally advanced squamous cell cancer of the head and neck in combination with 5-FU and platinum or with radiation.

Mechanism: A chimeric antibody (IgG1) that specifically binds to EGFR-1 on cell surface. KRAS signaling is downstream (internal in the cell) and mutations in KRAS cause activation of signaling independent of EGFR-1 signaling which occurs at the cell surface. See figure 2. (Same holds for panitumumab below.)

Toxicities: Severe immune/allergic reactions, cardiopulmonary arrest (black box warnings); rash, severe nausea, vomiting, and diarrhea.

Note(s): This is the drug that was being developed by the company that Martha Stewart invested in. Martha engaged in insider trading activity (sold her shares on inside information) when the company stumbled and hastily submitted sloppy data to FDA. FDA did not approve cetuximab (in 2001).

Figure 2. Schematic of ligand binding to EGFR-1 and subsequent signaling through KRAS.



Bevacizumab (Avastin); approved in 2004

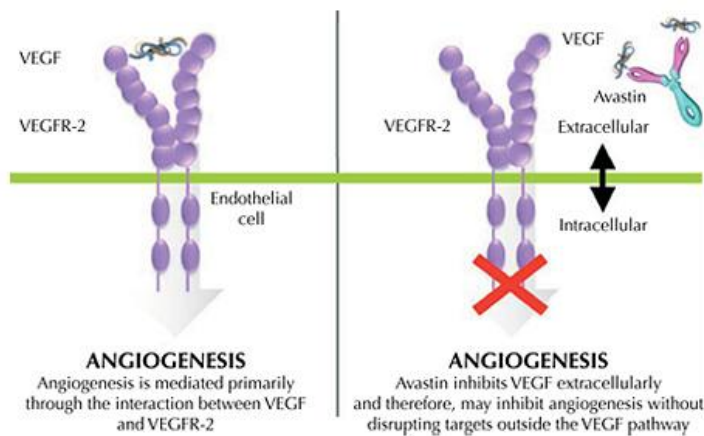
Uses: Colorectal cancer in combination with 5-FU based therapy (FOLFOX or FOLFIRI); non squamous non-small cell lung cancer in combination with carboplatin and paclitaxel; glioblastoma as a single agent; renal cell carcinoma in combination interferon- α .

Mechanism: A humanized antibody that binds to vascular endothelial growth factor (VEGF), which is the ligand that binds to the vascular endothelial growth receptor (VEGFR-2). See Figure 3.

Toxicities: Hemorrhage, GI perforations, stroke; cardiotoxicity especially with anthracyclines (all black box).

ADME: Elimination t_{1/2} about 20 days but can range (10-50 days).

Figure 3: Schematic of VEGF binding to VEGFR-2 (left) along with bevacizumab binding to VEGF (right).



Panitumumab (Vectibix); approved in 2006

Uses: Similar to cetuximab; for metastatic CRC, but as a single agent for the treatment of epidermal growth factor receptor (EGFR-1) expressing tumors*, with disease progression on or following fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens (i.e. FOLFOX or FOLFIRI).

Mechanism: A humanized antibody (IgG2) that specifically binds to EGFR-1 on cell surface.

Toxicities: Similar to cetuximab; still high incidence of dermatologic toxicity (rash) and immune/allergic reactions (black box warning). Cetuximab is chimeric but panitumumab is humanized.

ADME: Elimination t_{1/2} is about 8 days but can range (4-10 days).

Note(s): *Like cetuximab, panitumumab is not to be used for treatment of patients with KRAS mutation-positive mCRC or for whom KRAS mCRC status is unknown. KRAS signaling is downstream (internal in the cell) and mutations in KRAS cause activation of signaling independent of EGFR-1 signaling. Figure 2 above.

Ofatumumab (Arzerra); approved in 2009

Uses: Chronic lymphocytic leukemia (B-cell) that is refractory to fludarabine and alemtuzumab, so second or third line.

Mechanism: A humanized antibody (IgG1e) that binds specifically to the loop regions of the CD20 antigens on cell surface.

Toxicities: Immune/allergic reactions (no black box)*; cytopenias (esp. neutropenia), progressive multifocal leukoencephalopathy (PML), pneumonia, hepatitis B reactivation**.

ADME: Elimination t_{1/2} can range widely among patients: 2-60 days.

Note(s): *Infusion reactions less since humanized and they tend to decrease with continued dosing; **infections are actually quite common and can be of bacterial, viral, or fungal origin.

Pertuzumab (Perjeta); approved in 2012; expanded in Sept 2013 to include neoadjuvant

Uses: Metastatic breast cancer but only if HER2+, in combination with trastuzumab (Herceptin) and docetaxel. Used first-line in patients who have not received prior trastuzumab therapy or chemotherapy. Recently got expanded approved in early stage HER2+ breast cancer.

Mechanism: A humanized antibody that binds specifically to the cell surface receptor HER2 similar to trastuzumab but specifically at the dimerization domain. This inhibition of dimerization of the HER2 proteins, inhibits signaling internally in the cell.

Toxicities: Immune/allergic reactions, pulmonary edema, cardiotoxicity.

ADME: Elimination t_{1/2} about 18 days; independent of dose.

Note(s): Approval based on comparison trial that showed pertuzumab + trastuzumab + docetaxel was more efficacious based on progression free survival (PFS) than placebo + trastuzumab + docetaxel.

Ipilimumab (Yervoy): approved in 2011

Uses: Metastatic melanoma, unresectable; useful as single agent.

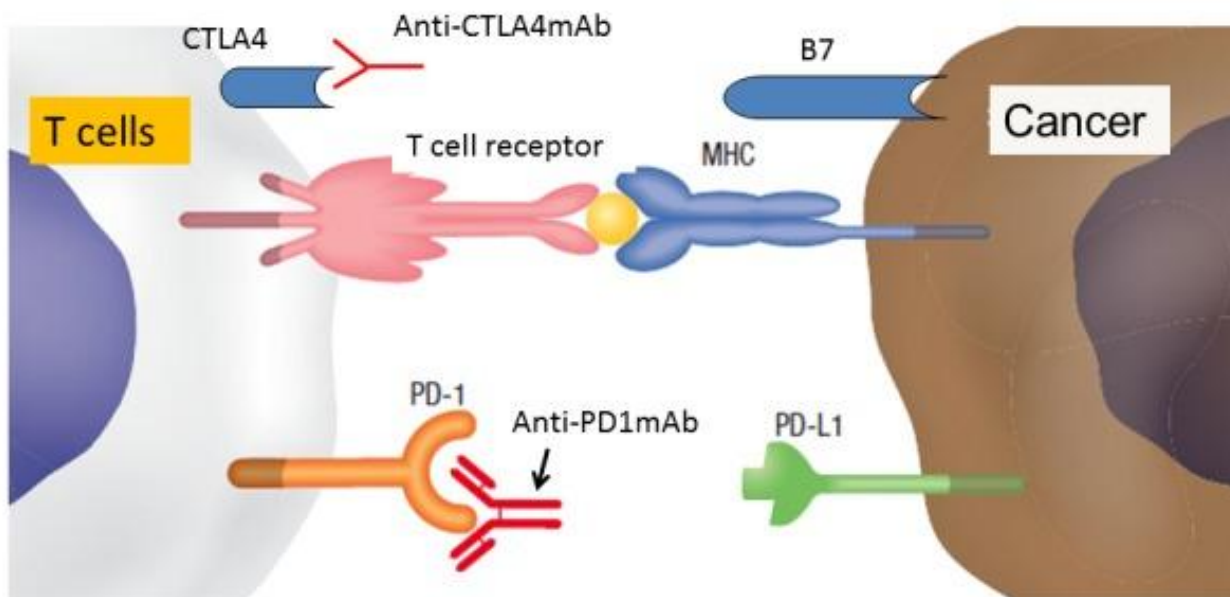
Mechanism: A humanized antibody (IgG1 kappa) that binds to the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). CTLA-4 is a negative regulator of T-cell activation*, therefore blockade of CTLA-4 will lead to T-cell activation and proliferation (see Figure 4). This enhances the immune response.

Toxicities: Severe and potentially fatal immune/allergic reactions due to T-cell activation (black box warning). Any organ system can be involved however, the most common are enterocolitis, hepatitis, dermatitis, neuropathy, and endocrinopathy. Most commonly manifest during treatment but can occur weeks or months later. **High toxicity but it works.

ADME: Terminal t1/2 of about 15 days; linear kinetics.

Note(s): *CTLA-4 is a T-cell “off switch”, while CD28 is the “on switch”. **Good example of high clinical hurdle and low regulatory hurdle. Although this agent is quite toxic, hardly anything else works for metastatic melanoma, so it was approved.

Figure 4. Some mechanisms of communication between T cells and cancer cells. Note that CTLA4 and PD-1 are negative regulators of T cells.



Pembrolizumab (Keytruda); approved 2014

Uses: Metastatic melanoma, unresectable; after failure of other agents such as Ipilimumab and if BRAF mutation V600 positive* (accelerated approval).

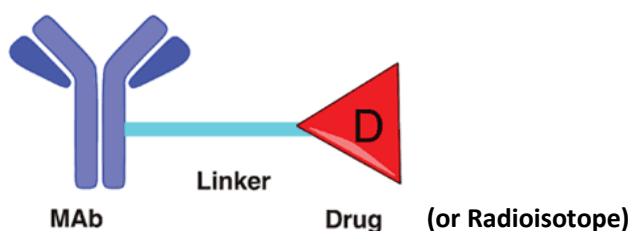
Mechanism: A humanized antibody that binds to the the PD-1 receptor and prevents binding of PD-L1. Leads to T-cell activation and proliferation (see Figure 4, above). This enhances the immune response.

Toxicities: The most common side effects are fatigue, cough, nausea, itchy skin (pruritus), rash, decreased appetite, constipation, joint pain (arthralgia) and diarrhea. However, potential for severe immune-mediated side effects (severe immune-mediated side effects involving healthy organs, including the lung, colon, hormone-producing glands and liver, occurred in clinical trials but uncommonly).

ADME: Terminal t1/2 of about 26 days; appears to have linear kinetics.

Note(s): *BRAF is a gene (proto-oncogene) that codes for the protein B-raf which is kinase (serine/threonine kinase). The V600 mutation is specifically (V600E), which is a change from valine to glutamic acid. The frequency of BRAF mutations in metastatic melanoma is about 50% and the frequency of these mutations being V600E is about 80-90%.

2. Antibody conjugates: combine antibody specificity with extra payload



Ibritumomab (Zevalin); approved in 2002

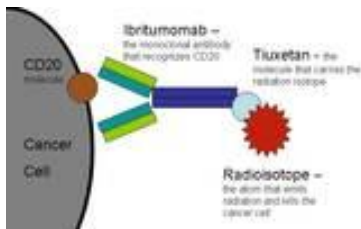
Uses: Relapsed or refractory, low-grade or follicular B-cell non-Hodgkin's lymphoma (NHL); previously untreated follicular NHL. Complicated procedure for use.*

Mechanism: Same antibody as in Rituxan that targets the CD20 antigen but conjugated to a radioisotope, Yttrium 90. See Figure 3. Yttrium is a β particle emitter and has a $t_{1/2}$ of 64 hrs** (about 3 days) so short lived.** Energy of 2.3 MeV.

Toxicities: Serious immune/allergic reactions, severe and prolonged cytopenias, and severe cutaneous reactions (all black box).

Note(s): *The therapeutic use of Zevalin actually includes the use of Rituximab first (Day 1). Rituximab is again on Day 7 and then followed by Zevalin 4 hrs later. **Because $t_{1/2}$ of isotope is so short it is important to calculate/confirm the radiochemical purity of the dose given.

Figure 5. Schematic of ibritumomab binding to CD20 antigen on a cancer cell.



Tositumomab (Bexxar); approved in 2003

Uses: Refractory B-cell non-Hodgkin's lymphoma after relapse following treatment with rituximab.

Note: Given in 2-step procedure involving a (1) dosimetric dose and a (2) therapeutic dose. The dosimetric dose is given to assess biodistribution and tumor burden in the body, as well as subsequent drop in platelet counts. Then based on information from the dosimetric dose, the therapeutic dose is calculated and administered.

Mechanism: Mouse antibody that targets CD20 antigen, so similar conjugate as Rituxan and Zevalin but incorporates different radioisotope (iodine 131; also a β particle emitter). Iodine 131 has $t_{1/2}$ of 8 days and energy of 971 KeV. Longer $t_{1/2}$ of iodine 131 easier to use than yttrium 90; specific activity of isotope not required.

Toxicities: Severe immune/allergic reactions, severe cytopenias (thrombocytopenia, neutropenia), infections, radiation exposure (all black box).

Note(s): Important limitation of use is that only one treatment can be given. This is the way the clinical trial was done, so this is the way it must be used. Additional complexities involved with dosing radioactive conjugates. Remember, FDA can review only data presented to them.

Brentuximab Vedotin (Adcetris); approved in 2011 (Seattle Genetics)

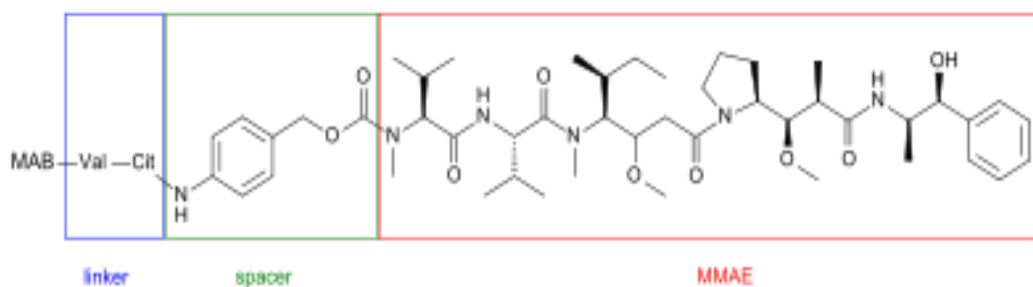
Uses: Hodgkin's lymphoma after failure of stem cell transplant ASCT or after failure of at least two prior multi-agent chemotherapy regimens in patients who are not ASCT candidates, and systemic anaplastic large cell lymphoma (SALCL) after failure of at least one prior multi-agent chemotherapy regimen.

Mechanism: A chimeric antibody-drug conjugate consisting of three components: 1) the chimeric IgG1 antibody specific for human CD30, 2) the microtubule disrupting agent MMAE*, and 3) a protease-cleavable linker that covalently attaches MMAE to the antibody. See Figure 6.

Toxicities: Progressive multifocal leukoencephalopathy (PML) secondary to JC infection** (black box warning); neutropenia, peripheral sensory neuropathy, fatigue, nausea, anemia, upper respiratory tract infection, diarrhea, pyrexia, rash, thrombocytopenia.

Note(s): MMAE is monomethylauristatin E (antimitotic) which is extremely toxic and failed in early clinical trials. MMAE is extensively metabolized by CYP3A4. Strong inhibitors or inducers can change the clearance of MMAE; **Immunodeficiency or immunosuppression allows JCV (John Cunningham virus) to reactivate. In the brain it causes the usually fatal PML by destroying oligodendrocytes. JC virus is very common in the human population (70-90% humans have it).

Figure 6. Structural components of brentuximab vedotin. In reality, there are about 4 MMAE molecules attached to each antibody molecule.



Denosumab (Xgeva); approved in 2010

Uses: For prevention of skeletal-related events related to bone metastases in patients with solid tumors. Not for multiple myeloma.

Mechanism: A human IgG2 monoclonal antibody that binds to human RANK ligand (RANKL). RANKL is a transmembrane protein involved in the formation, function, and survival of osteoclasts, the cells responsible for bone resorption. Thus, denosumab binds RANKL* and prevents it from activating its receptor, RANK, on the surface of osteoclasts. Increased osteoclast activity, stimulated by RANKL, is a mediator of bone pathology in solid tumors with bone metastases.

Toxicities: Asthenia, nausea, hypocalcemia, osteonecrosis of the jaw. No black box.

Note(s): Administer calcium and vitamin D to prevent hypocalcemia. Note this agent binds to the ligand of the receptor, not the receptor itself.

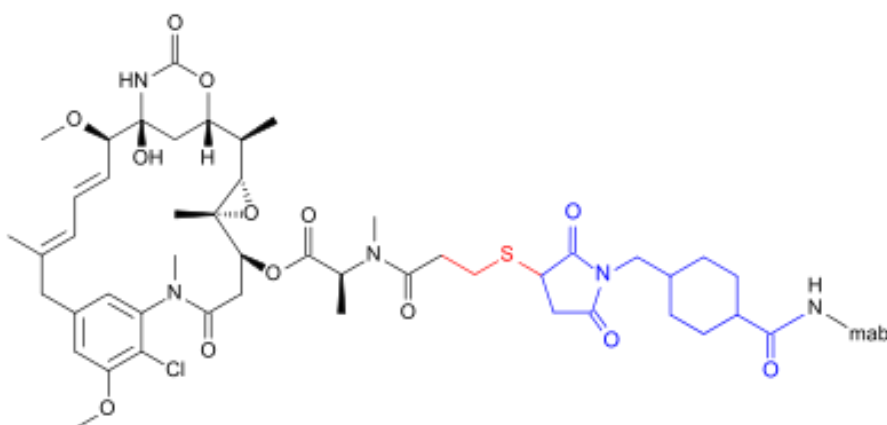
Trastuzumab Emtansine (Kadcyla); approved in 2013.

Uses: HER2+ breast cancer, following progression after treatment with trastuzumab + chemotherapy.

Mechanism: Combines the targeting specificity of trastuzumab with the cell killing power of emtansine (DM1). Emtansine (DM1) is an antimetabolic agent that prevents the assembly of microtubules.

Toxicities: Hepatotoxicity, cardiotoxicity, pulmonary toxicity, myelosuppression, fetal/embryo toxicity.

Note(s): HER2 testing required before use.



ZIV-Aflibercept (Zaltrap): approved 2012

Uses: Metastatic colorectal cancer (mCRC) in combination with 5-FU, leucovorin, irinotecan-(FOLFIRI), is indicated for patients with that is resistant to or has progressed following an oxaliplatin-containing regimen.

Mechanism: A fusion protein that binds to and inhibits the action of vascular endothelial factor (VEGF). It actually binds to several growth factors (VEGF-A, VEGF-B and placental growth factor PGF). Because of the importance of VEGF in cancer cell growth, this agent is being evaluated for use in several other types of cancer.

Toxicities: Hemorrhage, GI perforation, compromised wound healing (all black box warnings).

Notes: Also used for wet macular degeneration (actually approved for this use first).