Bevacizumab pharmacology and applications

Introduction

Avastin® (Bevacizumab solution for injection; Genentech, San-Francisco, Canada), is a humanised (93% human, 7% murine sequence) IgG 1 monoclonal antibody prepared by recombinant DNA technology. It is a Vascular Endothelial Growth Factor (VEGF) specific angiogenesis inhibitor. It belongs to the class of drug considered the fourth modality for cancer treatment. In 2004, it became the first angiogenesis inhibitor drug approved solely for cancer chemotherapy; firstly for the treatment of colorectal cancer, and later approved for other malignant conditions (1, 43).

Chemistry/composition

Typically, Bevacizumab is a monomer with molecular weight of 149KDa. It has 3 major fragments namely: Fv (Fragment variable of murine origin), Fab (Fragment antigen binding) and FC (Fragment crystallisable of human origin) which performs effector function. Each V (variable) domain contains 3 short stretches of peptide and hypervariable sequences (HV1, HV2 and HV3) known as the complementarity determining regions (CDR) – antigen binding region (30). The heavy chains show C-terminal heterogeneity (lysine variants) and also contain one N-linked glycosylation on asparagine at position 303. Two inter-chain covalent disulfide bonds couple the two heavy chains. Each light chain is covalently joined through a disulfide bond at cysteine 214 to a heavy chain at cysteine 226 (6).

Avastin comes as a lyophilised solid for reconstitution prior to use. The solid is a clear to slightly opalescent, colourless to brown, sterile; pH 6. 2 solution given by intravenous infusion. It is supplied in 100mg and 400mg

preservative-free single use vials to deliver 4ml or 16ml of Avastin (25mg/ml). The 100mg product is formulated in 240mg Î±, Î±-tetrahalose dehydrate (acceptable non-compendial specifications), 23. 2mg sodium phosphate (monobasic, monohydrate), 4. 8mg sodium phosphate (dibasic, anhydrous), 1. 6mg polysorbate 20, and water for injection USP. Its 400mg product contains the same amount of ingredients in the order of 4-folds. It can be stored up to 24 months at 2-8OC and given IV, mostly on days 0, 28, 35 and 42 or every 14 days (6).

Pharmacology

Tumor Angiogenesis

Angiogenesis is the formation of new blood vessels sprouting from the pre-existing vasculature. It occurs in normal and pathological conditions, including those associated with cancer (2, 9). The association of angiogenesis and cancer was initially discovered about fifty decades ago (11-13). Folkman et al., in 1971, first proved that angiogenesis was one of the major steps in tumor progression and metastasis (4, 14) shown in Fig. 2. Gullino showed that cells in pre-malignant tissues acquired angiogenic capacity which is dominant when malignancy is attained (15), which was confirmed through genetic studies, that acquisition of an angiogenic phenotype, was one of the hallmarks of cancer (3, 16-18).

VEGF A is the major angiogenic factor and regulator of tumor neovascularisation in humans. It enhances endothelial cell proliferation and blood vessel formation. Over-expression of VEGF in most tumors worsens the prognosis (8, 20). Avastin® explores the most efficacious of the three

possible mode of blocking VEGF’s activity (neutralise VEGF); others either block production (Iressa) or block receptors for VEGF and other angiogenic stimulators -Sutent (7).

Fig. 2. Tumour angiogenesis. Angiogenesis is initiated by the production of angiogenic factors from tumour cells, such as vascular endothelial growth factor (VEGF). Upon binding to its cognate receptors on endothelial cells, VEGF triggers endothelial cell proliferation and migration. Degradation and invasion of extracellular matrix (ECM) then follow. Endothelial cells assemble into a tubular structure. The process is completed by loop formation and vessel wall maturation. (EC = endothelial cell) – Ref-[7].

Pharmacodynamics:

Bevacizumab binds with high affinity, to all human VEGF-A with its Fab fragment, which interacts selectively with ligand VEGF (complement fixation), prior to VEGF’s connection to the natural endothelial receptor, which leads to antibody-dependent cellular cytotoxicity (ADCC). Normal ligand-receptor interaction is blocked; also receptor phosphorylation and downstream pathways are activated. Thus, vascularisation is regressed; tumor vasculature formation and tumor growth are also inhibited (23). It binds and inhibits the biological activity of humanVEGF during in vitro and in vivo assay systems (64). Preclinical in vivo tumor growth studies of Bevacizumab administration to xenograft and metastatic models of cancer in mice showed reduction of microvascular tumor growth and inhibition of metastatic disease progression (24, 25).

Pharmacokinetics:

In contrast to small molecule drugs, the typical metabolic enzymes and transporter proteins such as cytochrome P450 and multi-drug resistance efflux pumps are not involved in the disposition of monoclonal antibodies (mAbs) (62). Pharmacokinetic studies of Bevacizumab were conducted in mice, rats and rabbits given by intraperitoneal injection; absorption was complete which was slower for subcutaneous injection. Its distribution was assessed in rabbit model because recombinant mAb (rhuMAb VEGF) was discovered to bind to rabbit VEGF but not mouse or rat VEGF recombinant MAb (24).

This model showed that most of rhuMAb was retained in the plasma, with more spreading to the heart, testes, bladder and kidney in comparison with other organs (which justifies its treatment of cancer of such organs); suggesting that Endogenous antibodies and Avastin is similarly cleared and regulated by Brambell receptors (54). Its Pharmacokinetics was thus characterized as a 2-compartment model from earlier studies done. Patients who received doses ranging from 0. 1 to 10mg/kg intravenously over 4-24 weeks had an average clearance of 239mL/day, with an average Vd (volume of distribution) of 3260mL in the central compartment (6). Mechanism of metabolism and elimination has not yet been described in published data. However, results from a phase II trial showed that human clearance and half-life for Avastin was about 2. 79 mL/kg/day and 21days respectively (6).

Clinical applications:

It is used for the management of metastaic colorectal cancer, with intravenous interferon alfa or 5-fluorouracil-based chemotherapy for first- or

second-line treatment (31); non-squamous non-small cell lung cancer, with carboplatin and paclitaxel for first line treatment of unresectable, locally advanced, recurrent or metastatic disease (32, 34); first line therapy for metastatic HER2-negative breast cancer (33, 35); glioblastoma, as a single agent (intraarterially / intracranially) for patients with progressive disease following prior therapy; and macular degeneration (48, 56).

Side effects/Adverse effects:

Commonly reported effects are hypertension, proteinuria and haemorrhage. Less frequent adverse events include arterial and venous thromboembolic events (ATE, VTE), congestive heart failure, wound healing complications and gastrointestinal perforations. Generally, these adverse events are not dose dependent in any indication (with the exception for hypertension and grade 1 proteinuria). These Adverse events require habitual monitoring (hypertension, proteinuria); avoid overdosage (hypertension, VTE); temporary dose stoppage (hypertension, proteinuria, VTEs, wound healing) to absolute stoppage of regimen (for all life threatening events) – 38, 40-43.

Development/Formulation:

The first approved mAbs for therapy – orthoclone (OKT3) which was of murine origin, stimulated the formation of neutralising human anti-mouse antibodies which reduced the drug’s activity (via immunogenicity). Murine MAbs were engineered to the chimeric type (mouse CDR, human Fc) but still had the same limitations typified by rituximab; however, with a longer in vivo half-life (57, 62). Bevacizumab development (humanized Mabs) was meant to reduce the xenogenic portion, thereby limiting immunogenicity (23). Two

methods of achieving absolute biocompatibility of mAbs with humans (fully humanized mAbs), are phage display library (Adalimumab/Humira) and use of transgenic XenoMouse® – Panitumumab / Vectibix (63).

Bevacizumab is produced by recombinant DNA technology in Chinese hamster Ovary (CHO) cells, with gentamicin (antibiotic) as part of the expression system. Its manufacture is based on a CHO master and working cell bank system, which have been thoroughly characterised and tested to exclude harmful contaminants and endogenous viruses in accordance to ICH guidelines. Manufacture consists of series of steps which include fermentation, harvest and purification. Chromatographic and viral inactivation / removal procedures were combined to purify the product (45).

Structurally, it is large, complex, lipophilic and prone to degradation by acids and enzymes as well as temperature extremes and solvents (58, 69). The development of the product was intended to achieve a stable liquid intravenous formulation (as infusion), the most prominent means of delivery of antibody therapeutics, which is able to reach diffuse and inaccessible tumor sites (49, 64). Also, liquid formulations are cheaper, easily developed and easier to prepare for administration.

Its presentation as a liquid formulation was limited by aggregation and deamidation reaction (water-accessible regions) which could affect the product’s integrity / efficacy (65, 68, 75) by inducing charge heterogeneity detectable by isoelectric focusing or high-performance cation-exchange chromatography (66, 67). At low concentration, it adheres to container walls and exhibits aggregation at high concentrations. Concentration allowed will

not be adequate for clinical response in chronic therapy. Also, it is difficult for concentrations > 50mg/ml to pass through the needle guage due to its viscosity – solution dimerisation (70, 71). It justifies its administration by infusion. Other conditions that encouraged this situation were pH 6. 5-7. 5, IM NaCl (72, 73).

This challenge was partly managed by post-translational modification. i. e. N-linked glycosylation at asparagine 303 during manufacture of Avastin (45, 55, 71); even though, the antigen specificity of some therapeutics could be affected by substituting some amino acids of the Fv fragment (59-61). Also, solution formulation was lyophilised to reduce the impact of water (deamidation, adherence / aggregation and loss of potency) on Bevacizumab because it is stable to freeze drying in the absence of excipients that act as cryoprotectants (76-78). Its freeze drying with residual water content (1-8%) allows for optimal stabilization in the dry state and upon reconstitution, as confirmed by a recent study (22). Avastin was formulated with buffer (sodium phosphate – mono & dibasic), sugar (trehalose dihydrate) and detergent (polysorbate 20) after lyophilisation, to achieve a favourable pH for the product and reduce its aggregation rate (45, 74, 44). Î±, Î±-trehalose dihydrate are better anti-aggregation agents than sucrose (also induce acute renal failure), with higher Tg – glass transition temperature (39, 46).

The use of polymeric systems to improve stability of some products has shown inflammatory response (47). An exception is the case of intra-vitreal injection of Bevacizumab loaded PLGA microspheres for macular degeneration which provides sustained drug effect with fewer tendencies to immunogenicity (48).

Fab fragment of Bevacizumab for therapy may avoid the immunological reactions associated with whole antibody whilst improving the pharmacokinetics of the drug when conjugated with polyethenyleneglycol (PEG). Although, it has not been developed for clinical use; results from in vitro site-specific mono-PEGylation of Fab of Avastin showed that PEG-Fab (10kDa) binded in a similar manner as the native Fab (as analysed by Biacore). However, more studies would still be done to improve the binding affinity of feasible PEGylated Fab. Also, in vitro and in vivo studies on fabricated Avastin tissue tablet for subconjunctival implantation showed that such formulation would prolong drug release and improve outcomes of wound healing after glaucoma filtration surgery (37a, b).

Clinical data

Avastin has consistently being involved in several clinical trials that showed encouraging results. It is often combined in many phase II solid tumor trials, with erlotinib HCl, a tyrosine kinase inhibitor for synergism (5, 19, 21, 47, 50-52). Remarkably, a result of a phase II trial, multicentre, open-label, noncomparative study was recently published, where the efficacy of Bevacizumab, as a single agent and in combination with irinotecan, in recurrent glioblastoma was evaluated and results shown in Table1. Predetermined dose of the drug was given to 167 patients and objective response rate and 6-month progression free survival were the primary end points while safety and overall survival were the secondary end points. It was inferred from the results obtained that Bevacizumab, as a single agent or in combination with irinotecan was reasonably safe and effective in recurrent glioblastoma (53).

Conclusion

Bevacizumab’s versatility for the management of variety of malignancy is associated with the expression of VEGF in almost all tumor types (10, 15). Intravenous route /local delivery (intracranial/intravitreal) are the only feasible mode of delivery as other routes compromise drug efficacy/safety (79, 80). It is hoped that specificity of its Fab-PEG conjugate for the VEGF ligand in future clinical studies will reduce the bulk of Avastin for delivery whilst optimising therapy. Also, It has been proven that antitumor activity of Avastin® can be improved by its combination with chemotherapy (26), radiation (27, 28) and other antiangiogenic agents (29).

Avastin-resistant tumors have been managed by targeting other angiogenesis signalling pathways such as platelet-derived growth factor-C (36).