Defining and quantifying avidity of bispecific antibodies

Modeling spatial restrictions after primary binding and complex abundance on a population level

Author: Eva Lukas (7795936)

Supervisor: Prof. Dr. P.H. van der Graaf
Examiner: Prof. Dr. J.H.W. Leusen

Abstract

In the last decade, bispecific antibodies gained in attention due to their potential use in oncology. In contrast to their monospecific counterparts, bispecifics target two epitopes simultaneously offering a novel approach of antigen recognition. This may present a tool in overcoming resistances in oncology. Unique to bivalent antibodies is furthermore their ability to act as a bridge between cells of the immune system and cancerous tissue (trans-binding). While the concept of affinity of a single antibody arm to its target is clearly defined, the term of avidity is less distinctly described. We here present various perspectives of avidity and their elaboration in literature. We introduce mathematical models that aim to quantify deviations in binding strengths in bivalent antibodies as compared to the sum of their monospecific counterparts. As trans-binding antibody are affected by affinities in their localization and target specificity rather than in their strengths of binding, we focus on cis-binding antibodies having same-cell targets. The change in microenvironment upon primary target is modelled as a hemisphere. This concept is then included in population-based models explaining dynamics of complex formation. We conclude that avidity depends on many factors characteristic to the antibody and determined by the system. The change in environment and affinity after primary binding explains deviations of antibody dynamics from monoclonal mixtures and can be modelled on the molecular level (as hemisphere) or on a population level (introducing an avidity factor).

Lay summary

Antibodies are proteins that are part of the immune system. They recognize targets such as other proteins expressed on sick cells and initiate their clearance (Abbas et al. 2012). Commonly, the two antigen-binding arms of the antibody recognize the same target (monospecific antibodies). However, the antibody may also recognize different targets (Nisonoff et al. 1960). The combination of targets results in an antibody that acts differently from the sum of two monoclonal antibodies with the corresponding targets. These bispecific antibodies find application in oncology. Cis-binding antibodies can block multiple pathways, for instance involved in tumor progression or survival at the same time. This may prevent resistances of cancers (Labrijn et al. 2019). Alternatively, the different targets of an antibody may also be located on different cells (trans-binding). This allows to bring cells of different protein expression in proximity. Antibodies of any design bind to specific epitope noncovalently with a certain force, which is called *affinity* (Abbas et al. 2012). The binding strength of an antibody binding multiple different targets (termed *avidity*) on the other hand is less well defined. This report aims to provide an overview of definitions of avidity an introduces models to approximate the antibodies dynamics.

Upon binding of a cis-binding antibody to its first target, the antibody is locally restrained to where the proteins that it is bound to is located on the cell membrane. The antibody can bind to a second

protein if it is in proximity. The area which the antibody can reach after binding can be modelled as a hemisphere as wide as the distance of the arms of the antibodies (Crothers and Metzger 1972). With this in mind, we continued to gather models that allow to approximate the abundance of antibodies that have found both their targets. This can be done using ordinary differential equations. The likelihood of binding to a second target is multiplied with a factor that accounts for spatial restraints and proximity (*avidity factor)* (Harms et al. 2012). We conclude that while the likelihood of an antibody to undergo a second binding event may be unique to the antibody (Harms et al. 2012), systematic factors like target concentration play a role. Beyond simple models, further systems are introduced that include competition for binding between antibodies (Vauquelin and Charlton 2013), external molecule binding to one of the antigens (Vauquelin et al. 2014) and distribution of the drug into different compartments (Schropp et al. 2019). Avidity is thus an attribute of the mechanism of bivalent antibodies that depends on many factors. It alters the abundance and strength of antigen-antibody complexes. Ultimately, this results in clinical outcomes differing from monoclonal antibody administration. For trans-binding antibodies, binding of either antibody arm is largely independent of the other (Sengers et al. 2016). However, attraction to either target influences the movement and location of an antibody. For instance, one that bind T cells on one arm and tumor cells on the other may distribute to either lymphatic tissue or tumor-resident tissue, depending on the attraction to either target (Slaga et al. 2018). It is thus important to understand the role of affinity and the interplay thereof (avidity) to design optimal therapy. Future research may facilitate models summarized here to design antibodies that efficiently eradicate tumor cells while minimizing adverse health effects.

A. Introduction

I. Bispecific antibodies may be more than the sum of monospecifics

Originally discovered to be produced by tumor cells, monoclonal antibodies share their specificity. With the help of hybridomas, they can be modified to have a desired pathogen-specificity (Köhler and Milstein 1975). The use of monoclonal antibodies is various, including clinical diagnosis and therapy. Beyond mouse-antibodies, humanized antibodies are commonly used, containing the epitope recognizing region derived in mice and human antibody structured to minimize adverse immune responses to the administered antibody (Abbas et al. 2012).

An antibody may have more than one target (first suggested by (Nisonoff et al. 1960) and extensively reviewed by among others (Kontermann and Brinkmann 2015), (Labrijn et al. 2019)). These so-called bispecific antibodies (BsAbs) were first engineered using rabbit antibodies of either target, yielding antibodies that combine the targets of the initial material into one antibody (Fudenberg et al. 1964). In 1985, antibodies enabled redirection of T cells to attack specific cells using bispecific antibodies, binding the target on one side and a (cytotoxic) T cell (e.g. through T3) on the other (T-cell engaging antibodies; T-BsAbs) (Perez et al. 1985; Staerz et al. 1985).

The mechanism of BsAbs may not be the sum of effects of corresponding monospecific antibodies (Labrijn et al. 2019), possibly improving target specificity (Fan et al. 2015). Depending on the targets of antibodies, their binding is categorized. For targets located on different cells, an antibody may act to bring the epitope-harboring cells in proximity (*trans-binding*) (Labrijn et al. 2019); a special feature of bispecific antibodies compared to mixtures of monospecific (Fan et al. 2015). These antibodies may redirect T cells, targeting lymphocytes on one arm (often $CD8^+$ T cells) and tumor cells on the other. Alternatively, targets of bispecific antibodies can be located on the same cell (*cis-binding*). In oncology, this principle is for instance used to target more than one receptor tyrosine kinase, thereby avoiding resistances (Labrijn et al. 2019).

Cis-binding bispecific antibodies

BsAbs targeting multiple same-cell-residing receptors are extensively reviewed in literature (e.g. (Labrijn et al. 2019) or (Betts and Graaf 2020)). Current examples include Zenocutuzumab, JNJ-61186372 and XmAb23104 (Labrijn et al. 2019). Zenocutuzumab targets HER2 on one arm and HER3 on the other one. At the time of report (February 2022), phase I/II of Zenocutuzumab is still ongoing in tumors showing NRG1 fusion (NCT02912949 2022). Amivantamab and Lazertinib (= JNJ-61186372) are currently in phase II (NCT04965090 2022). XmAb23104 is still in Phase I (NCT03752398 2022).

T cell engaging bispecific antibodies

CD3- and CD19 bispecific antibodies (here notated as CD3 x CD19) did not show severe toxicities in clinical studies (study of six patients, maximum grade II toxicity), however activation of (cytotoxic) T cells was also limited (Gast et al. 1995). Blinatumomab, also a CD3 x CD19 bispecific, showed (partial) tumor elimination in 11 of 38 patients participating in the study. Thereby, concentrations exceeding 0.015 mg/m^2 daily for various durations showed effects on tumor size in different tissues. Adverse effects at stage III and IV were observed (Bargou et al. 2008). Blinatumomab was subject to phase II trial in 2012/2013 in 189 acute lymphoid leukemia patients (refractory or relapsed). A high fraction of patients reportedly did not show evidence of the disease anymore or stabilized blood counts in the periphery (33% and 10% respectively in first 2 cycles, 4 weeks each). Patients of these categories survived without relapse for 5.9 months (6.1 months for whole cohort) (Topp et al. 2015).

T-BsAbs can also target other tumor-associated antigens (TAA). One such possibility is the specific binding of HER2 on one arm, while targeting T cells via CD3 on the other arm. The antibody 4D5 IgG TDB is an example therefor. It was shown to be very efficient in the sense that few successful complex formations are required to awake the desired T cell cytotoxicity. However, healthy cells also express the growth factor HER2. This may result in adverse side effects (Junttila et al. 2014).

Another example are OC/TR F(ab')2 antibodies, which were designed to target T cells and ovary tumor cells. Dose correlating toxicity was observed (grade III and IV), likely caused by stimulated increased cytokine release for concentrations > 0.1 mg. (Tibben et al. 1996).

II. The well-defined concept of affinity describes the strength of epitope binding

Antibodies bind to specific epitope noncovalently with various strengths (*affinity*) forming an "antigen-antibody complex" (Abbas et al. 2012). The concept of affinity is determined by characteristics of the drug only and may thus be referred to as a *Chemomeasure* (Agoram and Graaf 2012). Considering a binding event of a monovalent antibody (Ab) and its antigen (Ag) depending on the rate of complex ($AbAg$) formation (k_{on}) and on the rate of loss of binding (k_{off}), the dissociation constant of the complex (k_d) is defined as k_{off} over k_{on}. The smaller k_d, the higher is the affinity. The dynamics of the complex are then easily described as (Lauffenburger and Linderman 1996):

$$\frac{d[AbAg]}{dt} = [Ab][Ag]k_{on} - [AbAg]k_{off}$$

The rates of complex formation and bond breaking (k_{on} and k_{off}) for monoclonal antibodies can be calculated from experimental data. One such method is BIAcore. It notes interactions between fixated antigens and flowing antibodies. Complex formation over time and over various concentrations serves to obtain k_{on} and k_{off} (reviewed by (Malmborg and Borrebaeck 1995)). Similarly, kinetic exclusion assay (KinExA) measures unbound educt using fixed binding counterparts and

fluorescent emission (reviewed in (Darling and Brault 2004)).

The dissociation constant of the complex formed between a ligand and a target (k_d) can be assessed with the Scatchard plot (introduced by (Scatchard 1994) reviewed by (Lauffenburger and Linderman 1996)). Complex abundancy in equilibrium is plotted against the ratio of complex and free ligand in equilibrium. The slope of the resulting linear plot gives the dissociation constant (Lauffenburger and Linderman 1996). Note that this model holds for monovalent binding to one ligand. The dissociation constant can also be calculated by the concentration of complex over the concentration of free antigen (-harboring cell) times the concentration of free antibody ($[AgAb]/[Ag][Ab]$) (Crothers and Metzger 1972).

Affinity in bispecific antibodies

Beyond affinity, the last decade was marked by *avidity* as a term to describe binding characteristics of antibodies to multiple targets. This literature review aims to give an overview of definitions and quantifications of avidity in bivalent mono- and bispecific antibodies. We explore the origin of changes in binding affinity after dimer formation and introduce current models that capture these dynamics on a population level.

A. Defining avidity

Avidity of a population of antibodies is used to describe the overall effective tendency to bind a single target as a result of a variety of antibodies bearing different affinities (Pullen et al. 1986). The antibodies involved bind to either the same or various targets multivalently. While each binding event has its affinity when binding monovalently, the overall strength of binding of the antibody-antigen complex may not equal the sum of affinities. This phenomenon is termed *avidity* (Abbas et al. 2012; Vauquelin et al. 2014; Rhoden et al. 2016), or *functional affinity* (Karush 1970).

III. The origin of avidity in cis-binding antibodies is a change in environment after binding

In monospecific bivalent antibodies, binding of an antibody to multiple of the same rather than only one molecule increases its association constant. This may allow lower concentrations of a certain antibody to amount to comparable responses of single-target binding (monovalent antibodies) (Karush 1970). Furthermore, upon primary binding, the environment of an antibody is altered, possibly improving the chance of second binding (Crothers and Metzger 1972; Kaufman and Jain 1992; Lauffenburger and Linderman 1996; Harms et al. 2012). This is accompanied by steric hindrances upon primary binding, including the size and rotational freedom of the antibody (Crothers and Metzger 1972; Kaufman and Jain 1992; Harms et al. 2012). One may want to quantify the net-effect by a so-called *avidity constant*. Independent determination thereof is difficult, as among others ligand concentrations play a big role (Lauffenburger and Linderman 1996).

Avidity can also be explained by the likelihood of an antibody to establish a second binding depending on spatial changes as described above. While complex dynamics depend on conditions such as epitope abundance, the tendency to amount more binding after antigen binding is a constant characteristic of an antibody (Harms et al. 2014). Avidity may otherwise be explained as an effect, rather than as a concept or constant. The binding of a primary target may facilitate the binding of the second target. Within the time that the antibody is kept in a dimer with the first antigen, a second antigen undergoes Brownian motion. The maintenance of the antibody at the position of the first antigen thereby enables the second target to be found. This also holds in cases where antigens are technically too far away from each other to be bridged by one antibody (as reviewed by (Vauquelin and Charlton 2013), discussed

below). This observation is termed the *Avidity effect* in literature (Sengers et al. 2016).

IV. Avidity in trans-binding antibodies

When regarding T-BsAbs it is accepted that binding events happen independently as two different soluble targets are involved (Sengers et al. 2016). However, different affinities of antibody arms influence the delocalization of the administered antibody (Slaga et al. 2018). This phenomenon amounts to an observed effect differing from the application of two different monospecific with the according targets. This concept is here also referred to as avidity.

The difference in definition of avidity has an influence on its property. Defining avidity as an observed affinity due to different affinities within the antibody pool (Pullen et al. 1986) and in overall binding strength due to abundance of mono- and bivalent target binding (Kaufman and Jain 1992) causes avidity to depend on experimental settings. This poses a very different identity on avidity than when it is regarded as a biophysical constant determined by the antibody itself (Harms et al. 2014).

V. Avidity as a tool in clinical application

Knowledge about avidity of a bivalent antibody is relevant to predict its clinical behavior. By designing one arm of a cis-binding antibody to have high affinity, the binding of the second arm to a receptor that is found at lower concentrations may be aided (e.g. PB4188; HER2 x HER3) (Labrijn et al. 2019). Quantifying the rate of cross-linking after primary binding (avidity factor, elaborated below) and simulating complex formations revealed that monospecific bivalent antibodies possessing higher avidity factors are more sensitive to variations in antibody concentrations. As a certain threshold is exceeded, dimers are formed rather than tetramers (Harms et al. 2012).

For trans-binding antibodies, gradients in affinity may determine the spatial distribution of the drug. Examples thereof are CD3 and tumor targeting antibodies. They locate to lymphatic tissues (spleen, lymph nodes) depending on the affinity of the CD3 binding arm. The tumor targeting arm on the other hand allows for localization to tumor-resident tissue. As these effects interfere with each other, the affinities of the binding arms may be tuned for best performance. Lower CD3 affinities allow better distribution to tumor sites (Mandikian et al. 2018). When maintaining $CD8^+$ T cell activation, tumor killing is thereby more efficient as compared to their counterparts having higher CD3 affinity (Bortoletto et al. 2002).

Tuning the affinity of the binding arms targeting tumor cells may provide a strategy to avoid the involvement of healthy tissue. Decreasing the binding affinity of the tumor targeting arm can lower the risk of HER^{low} cells being bound. However, the affinity to HER2 is determining for the efficacy of the drug measured by activated T cell response. Avidity can help here, by providing overall binding strength through bivalent low-affinity binding. While HER^{low} cells are thereby unlikely targeted, T cell activity is established towards tumorous cells (Slaga et al. 2018).

B. Quantifying avidity

Many experimental factors influence the observed avidity of an antibody and its targets, making mathematical models attractive tools to predict, explain and analyze the antibody-antigen complex abundance. As ternary complexes built between both antibody arms and their targets are regarded as pharmacologically active (Betts and Graaf 2020), it is of interest to explore effects of designed affinities, concentrations, and other attributes with minimal experimental expense. In the following we will first focus on models established for cis-binding antibodies. As formation of a dimer causes the antibody to be cell-bound, conditions for binding to the second target are altered. This can be modelled by

assuming the dimer to be able to bind within its reach. This concept is then considered on a population level of complexes and their constituents. We will then return to avidity in T-BsAbs as defined above. Key points of avidity and its quantification in cis- and trans-binding antibodies are summarized in Figure 1.

VI. The accessible area of the antibody-antigen dimer can be modelled as a hemisphere, determined in its size by the reach of the antibody

In 1971, DeLisi and Crothers modelled the spatial location and orientation required for reaction between bases within a double DNA strand (DeLisi and Crothers 1971). In the following year, this model was further developed for the use of binding events between antibody binding sites and their epitopes. The formation of a ternary complex (both binding sites and both antigens) is dependent on circumstances established by the first binding. Thereby, binding arms for target one ($Ag1$) and two ($Ag2$) are separated by an average distance r. Secondary bond formation depends on the distance and spatial orientation (angular) of the second binding arm of the antibody and the second target. However, in the case of targets found on the same cell (cis-binding), rotational orientation of the second target is regarded negligible. Logically, the antibody is more likely to also bind the second target after primary binding if the second arm and epitope are in proximity (largest entropy preservation). Upon binding, the antibody reaches to its second target within a sphere surrounding the antigens location on the membrane. Mathematically, the equilibrium constant of the second binding can be modelled using the equilibrium constant of the first dimer ($K_1 = \frac{[AgAb]}{[Ag][Ab]}$), the number of antigen-containing cells (N), the volume of the experimental setting (V), and r (Table 1) (Crothers and Metzger 1972).

Continuing the model of (Crothers and Metzger 1972), (Perelson et al. 1980) investigated antibodies undergoing two valent bindings as depending on the (average) distance of the target binding arms (r) and the hemisphere surrounding the primary dimer. Primary attachment of the antibody to an antigen occurs at rate k_1. The second target must be within reach of the antibody when bound to a primary target already. It is bound at rate k_2. The concentration of epitope available to be bound ($[Ag]_{accessible}$) is obtained by dividing the area surrounding the antibody-antigen complex (πr^2) and the volume of the hemisphere ($\frac{2}{3}\pi r^3$) times the free epitope concentration (Perelson et al. 1980):

$$[Ag]_{accessible} = \frac{3}{2r}[Ag]_{free}$$

Assuming both targets to have the same association rate ($k_1 = k_2$) and considering that $K_x = {k_x}/{k_y}$ for x and y 1 and 2 interchangeably, one can solve for the equilibrium constant of the second binding event (K_2) (Table 1) (Perelson et al. 1980). Mind, that this assumption may not be valid for bispecific binding.

Kaufman et al (1992) further developed the model introduced by Crothers and Metzger (1972) as reviewed by Perelson et al. (1980). The often-required surface concentration of the antigen ($X_{Surface}$) of interest can now be calculated from the bulk concentration (X) using the radius of cells the antigen is expressed on (r_{cell}):

$$X_{Surface} = \frac{X}{4\,\pi\, r_{cell}^2\,(\frac{no.\,of\ cells}{[cm^3]})}$$

This surface concentration is then used for the mathematical expression of the concentration of the antibody, antibody-antigen dimers and tetramers between both binding arms of the antibody and according antigens (Kaufman and Jain 1992) (discussed below).

Table 1 Models on equilibrium constants of second-target-binding as dependent on changes in local environment of the antibody after primary binding.

Reference	Model	Explanation
(Crothers and Metzger 1972)	$K_2 = \frac{3}{2\,\pi\,r^3}\,\frac{V}{N}\,K_1$	The equilibrium constant of second binding depends on changes in the environment of the antibody upon primary binding. In successful binding events, second targets are found within sphere surrounding antibody-antigen dimer.
(Perelson et al. 1980)	$K_2 = [Ag]_{accessible}\,K_1$ $[Ag]_{accessible} = \frac{3}{2r}[Ag]_{free}$	The unbound arm of the monovalently bound antibody can reach the second target within hemisphere surrounding the dimer.

VII. Modeling monospecific bivalent antibodies

Using simple mass action dynamics, a maximum number of receptors available to be bound (a) and a number of receptors to be bound that are not internalized or otherwise inaccessible (b) ($a > b$), one can follow (Perelson 1981) while assuming a maximum number of possible bonds to be two. We consider a monospecific bivalent antibody who's binding to its antigens is reversible. Primary binding occurs at rate k_1 and depends on target expression on the cell membrane. k_2, or higher when more targets are involved, is defined to be a "cross-linking constant". The change in antigens, antibodies and complexes eventually depends on the participants concentration, the number of available or accessible targets as well as their association constants with the antibody (Table 2) (Perelson 1981).

Harms et al., (2012) also investigated bivalent monospecific antibodies and introduced a model based on Perelson 1981 and Crothers and Metzger 1972. The association constants to the targets differ depending on the order of association. To obtain parameters feeding the model, the binding of a monospecific monovalent interaction is measured (k_{on}). The first target is bound with $2\,k_{on}$, while the second target is bound with k_{on} times a so-called avidity factor (here called x) (Table 2). This factor is determined by the change in microenvironment of the antibody once it is bound to the first target. The change in environment depends on the reach of the antibody within the hemisphere surrounding the dimer as discussed above (Table 1, Perelson et al. (1980)). Here, the antibody concentration is not assumed equal throughout the medium (in contrast to (Sengers et al. 2016)). To allow this, the antibody concentration and association rates are converts to represent one cell worth of abundance (Harms et al. 2012):

$$[Ab]_{cell} = [Ab]_{molar}\,N_{av}\,V_{extracellular\ fluid}$$
$$k_{on_1} = 2\,\frac{k_{on}}{N_{av}\,V_{extracellular\ fluid}}$$
$$k_{on_2} = x\,\frac{k_{on}}{N_{av}\,V_{extracellular\ fluid}}$$

All concentrations and association rates can be experimentally measured (elaborated in detail in (Harms et al. 2012)), allowing for the

avidity factor x to be estimated as the only unknown parameter using computational simulations. Using different cell lines, large variations in avidity factor were seen, suggesting individual extends of avidity enhancement per antibody, possibly depending on the epitope the antibody is specific for (Harms et al. 2012). Looking at (Perelson et al. 1980) in (Table 1) one finds that the observation of the avidity factor to be characteristic to the antibody of interest can be conditionally confirmed. The accessible concentration of the epitope depends on the reach of the specific antibody and alters the binding affinity. However, the concentration of the epitope also plays an important role. Harms et al (2014) argue that the likeliness to undergo a second binding remains alike while changes in epitope concentrations effect the complex abundance (Harms et al. 2014).

(Vauquelin and Charlton 2013) agree with (Harms et al. 2012) in their modelling of association to the first target ($2\,k_{on}$). This is since antibodies that have the same binding properties to the same molecule on different arms have twice the probability and thus twice the rate to bind to the fitting molecule. However, these homospecific bivalent antibodies may not undergo two binding events if targets are too far away from each other (Table 2) (Vauquelin and Charlton 2013). Parameters to this model agreeing with experimental data would reflect an example of avidity not being enhanced by multivalency.

However, the effect of spatial lock and thus change in environment on affinity is usually positive. A higher avidity factor allows for a steeper response of binding as a function of antibody concentration. At lower antibody concentrations, binding is dominated by bivalent interactions (tetramer formation). The influence of the avidity factor and its improvement of binding outcome furthermore depends on the epitope concentration. High abundance of the target limits the effect of improvements in binding of the second antibody arm. Similarly, high concentrations of the antibody itself results in dimer formation predominantly (Harms et al. 2014).

Table 2 Models capturing the abundance of monospecific bivalent antibodies and their targets

Reference	Model	Explanation
Adjusted from (Perelson 1981)	$\frac{d[Ab]}{dt} = -a\,k_{on_1}[Ab][Ag] + k_{off_1}[AbAg]$ $\frac{d[AbAg]}{dt} = a\,k_{on_1}[Ab][Ag] - k_{off_1}[AbAg] - (b-1)k_{on_2}[AbAg][Ag] + 2\,k_{off_2}[AbAgAg]$ $\frac{d[AbAgAg]}{dt} = (b-1)k_{on_2}[AbAg][Ag] - k_{off_2}[AbAgAg]$	a is the abundance of target, b represents the target that is accessible. While both antigens have the same binding affinity to the antibody, complex formation depends on target abundance. Similarly, dissociation rates are equal for both antigens, however the chance to dissociate is double when two antigens are bound. No simultaneous release of both targets.
(Kaufman and Jain 1992)	$[Ab] = \frac{[Ab_T]\,(1-nr)}{1 + 2K_1[Ag] + K_1^2\,\Psi\,[Ag]^2}$ $[AbAg] = 2K_1[Ab][Ag]$ $[AbAgAg] = K_1^2\,\psi\,[Ab][Ag]^2$ with $\psi = \frac{3}{8\,\pi\,r_{cell}^2\,d\,(\frac{no.\,of\ cells}{cm^3})}$	The concentrations of antibody and complexes as dependent on their equilibrium constant. The formation of tetramers is determined by cell surface concentrations in proximity of the dimer. Nr notes the non-reactive antibody fraction.

(Harms et al. 2012) based on (Crothers and Metzger 1972; Perelson 1981)	$\frac{d[Ab]}{dt} = -k_{on_1}[Ab][Ag] + k_{off_1}[AbAg]$ $\frac{d[Ag]}{dt} = -k_{on_1}[Ab][Ag] + k_{off_1}[AbAg] - k_{on_2}[AbAg][Ag] + k_{off_2}[AbAgAg]$ $\frac{d[AgAb]}{dt} = k_{on_1}[Ab][Ag] - k_{off_1}[AbAg] - k_{on_2}[AbAg][Ag] + k_{off_2}[AbAgAg]$ $\frac{d[AbAgAg]}{dt} = k_{on_2}[AbAg][Ag] - k_{off_2}[AbAgAg]$	Association and dissociation rates are experimentally determined (k_{on}, k_{off}). $k_{on_1} = 2k_{on}$, $k_{off_1}. = k_{off}$ $k_{on_2} = x\,k_{on}$, $k_{off_2}. = 2k_{off}$. x accounts for the change in binding affinity due to change in environment of antibody upon first antigen binding. Off rates are unaffected by order of binding.
(Vauquelin and Charlton 2013)	$\frac{d[Ab]}{dt} = -2k_{on_1}[Ab][Ag] + k_{off_1}[AbAg]$ $\frac{d[Ag]}{dt} = -2k_{on_1}[Ab][Ag] + k_{off_1}[AbAg]$ $\frac{d[AbAg]}{dt} = 2k_{on_1}[Ab][Ag] - k_{off_1}[AbAg]$	Here, the antibody is thought to bind to one antigen only as they are too far away from each other to both be bound. In [AbAg], one binding site is thus free while only one site binds to the target.

Bispecific bivalent antibodies

The model introduced by Harms et al. (2012) as explained above can also be facilitated for the modeling of bispecific antibodies. Thereby, the avidity factor x reflects the change in secondary binding affinity as compared to primary binding events of a bivalent (possibly bispecific) antibody. The observed change in binding strength however also depends on the expression level of the targets. The more abundant a target, the less influential is the change in environment of the antibody after primary binding and spatial constrains reflected by x. x was found to determine the activity of a HER2/HER3 bispecific antibody, as measured by inhibition of the formation of HER2/HER3 dimers (x of tested antibodies ranged between $1\,x\,10^5$ and $5\,x\,10^3$). From this investigation, MM-111 resulted (completed Phase I in Breast Neoplasms, Metastatic Breast Cancer, other HER2 amplified (solid) tumors. Phase II was not completed as no significant effect could be determined (ClinicalTrials.gov 2022)). Moreover, using the model depicted in Table 2, the improvement of achievement of therapy goals of the bispecific antibody (HER3/IGF-1R) over monospecifc counterparts could be confirmed for the antibody termed MM141 (Phase II for pancreatic cancers concluded (NCT02399137 2022)) (Harms et al. 2014).

In 2016, Zheng et al. used target concentration on cell membranes and separately measured affinities to the different targets and binding data to fit the model of Harms et al. (2012) (Table 2) to estimate x for JNJ-611862372 (Janssen Pharmaceuticals). This BsAb binds both EGFR and c-Met on dedicated arms and showed an avidity factor of $x = 104$. This effect reflects spatial influences on the dynamics of the second binding after first-target binding. Especially so when there is a greater difference in affinity and/or abundance of the different targets. Binding to the first target may facilitate the binding to a less abundant or less prone-to-be-bound target (Zheng et al. 2016).

Rhoden et al. (2016) developed a set of ordinary differential equations that describe dynamics of the concentrations of the bispecific antibody recognizing MET and EGFR (cis-

binding). Thereby, antigens have different association and dissociation rates for primary binding which depend on antigen concentration. Secondary binding depends on antigen concentration once the bispecific is bound to one of its targets, and on the ratio of initial and current concentration of the antigen to be bound in second place (Table 3). k_{on} and k_{off} rates are measured by BIAcore (introduced above). Due to proximity establishment to the second antigen, the target is possibly bound at a higher rate than one would suspect from its concentration (Rhoden et al. 2016). The concentration of the second antigen when the antibody is in complex with the first target already (termed effective concentration in (Rhoden et al. 2016)) is here replaced by the antigen concentration and factors x_{Ag1} and x_{Ag2}. This allows to see parallels with the avidity constant introduced by (Harms et al. 2012).

Sengers et al., (2016) quantically investigated DuetMab, binding bispecifically to CD4 (high affinity) and CD70 (low affinity). The concentration of antibody is assumed to be constant and determines the abundance of binding states. When higher, more binary complexes accumulate. The concentration of available antigens is presented as the difference between antigens that are bound in a complex and the total abundance. The covalent binding to a second target of a dimer is supported by a factor x (Table 3). It represents the avidity effect that allows the association to the second target since the dissociation from the first target is slower than the Brownian movement of and the binding to the second target. Mind, that in contrary to (Rhoden et al. 2016), this effect is equal for both antigens and is thus independent of their individual concentrations.

For DuetMab this means that binding to CD4 due to higher affinity facilitates the binding of CD70 despite low affinity since the antibody dissociates slower from CD4 than a bond to CD70 is built. This is explained by the Brownian motion to be faster than the dissociation. Binding of both targets simultaneously is excluded (Sengers et al. 2016).

Competition between antibodies

Ideally, targets are within the reach of one antibody, thus the target molecules are apart by equal to or less than the span between the binding sites of the antibody. As one target is bound, the next one needs to lie within that distance (3 dimensionally). The concentration of that target within this reach may be higher compared to that in the solution which an unbound antibody is exposed to. While this is expected to increase the binding rate to the second target, steric restrictions may limit this effect. Vauquelin and Charlton (2013) built a model based on research of Kaufman and Jain (1992) and the review by Plückthun and Pack (1997) introducing a divisor for the association, similar to above models (Vauquelin and Charlton 2013). To allow for a comprehensive comparison between this and earlier models (Harms et al. 2012; Rhoden et al. 2016; Sengers et al. 2016), the divisor is here represented as a factor x (Table 3). As a novelty of this model, the possibility is introduced that a second target in proximity of an antibody-antigen dimer is bound to by another antibody ($[AbAg1|Ag2Ab]$), thereby introducing competition between antibodies. Neither the association nor the dissociation of bonds involved in this construct are determined by steric hindrances as described by x (Table 3) (Vauquelin and Charlton 2013). Thus, avidity effects are expected alike for both antigens.

Breaking the avidity factor down into change in a-/dis-sociation and altered antigen concentration

Rather than explaining all effects within a single factor x, a later model introduced to break down the effect of spatial lock upon dimer formation into a change in association and dissociation (α and α' respectively) rates and the concentration of the target in the new microenvironment of the dimer ($[L]$). α may include confirmational changes of the antigen(pair) influencing binding to a second

antigen in proximity. Contrary to previous models, effects of binding to a first antigen on the second antigen binding is not exclusive to association. $[L]$ represents the concentration of second target within the reachable hemisphere of the single-bound antibody (Vauquelin et al. 2014).

While the presence of two antibodies binding to a pair of antigens is not denied, they are removed from the model as their abundance is considered neglectable. Instead, a molecule different from the antibody (c) is competing for the first antigen. Binding of c may introduce confirmational changes affecting the binding affinity of an antibody to the second antigen (thought to be in proximity). Changes in association- and dissociation rates are then notes with α_c and $\alpha_c{}'$ respectively (Vauquelin et al. 2014). The introduced model is slightly altered by separating targets one and two in the set of equations. This allows for a better comparison with other models (Table 3).

Introducing compartments

Closer to reality, the administered drug does not need to be limited to be in one compartment only. One may want to consider a central- and a peripheral compartment. A general model of receptor-ligand binding as determined by ligand migration between two compartments was introduced by (Mager and Jusko 2001) and reviewed by (Dua et al. 2015). Translating the ligand to be an antibody and the receptor to be an antigen we read from the model that the antibody (Ab) is eliminated at rate k_{el}. It leaves into the periphery at rate k_p and returns with k_{-p}. As previously, antibody-antigen complexes are formed, here at a rate k_{on}, and are broken at rate k_{off} (Dua et al. 2015):

$$\frac{d[Ab]_{Central}}{dt} = k_{off}[AbAg] + k_{-p}[Ab]_{Periphery} - k_{on}[Ab]_{Central}[Ag] - k_{el}[Ab]_{Central} - k_p[Ab]_{Central}$$

$$\frac{d[Ab]_{Periphery}}{dt} = k_p[Ab]_{Central} - k_{-p}[Ab]_{Periphery}$$

Similarly, (Schropp et al. 2019) investigated the distribution of bispecific drugs in two compartments. The fraction of bispecific antibody in the drug (k_a) is distributed over the whole volume (V). Antibodies may also be administered intra-venous ($In_{iv}(t)$) into the central compartment directly. The drug may exit towards the periphery at rate k_P (return at rate k_{-p}) or be eliminated (k_{el}). In contrast to previous models, each epitope (Ag_1 and Ag_2) is synthesized (at rates $k_{syn_{Ag1}}$ and $k_{syn_{Ag2}}$ respecitvely) and degraded (at rates $k_{deg_{Ag1}}$ and $k_{deg_{Ag2}}$ respecitvely). Dimers and tetramers ($AbAg1, AbAg2, AbAg1Ag2$) are internalized at specific rates ($k_{int_{AbAg1}}, k_{int_{AbAg2}}, k_{int_{AbAg1Ag2}}$). The model requires 19 parameters (Table 3). Considering high affinity and resultingly very fast binding (quasi-equilibrium), one gets rid of five parameters. Furthermore, assuming the abundance of antigens not to change throughout antibody interference, 10 parameters remain to be estimated. The model including both assumptions allows for a simulation and prediction of an administration plan resulting in maximal ternary complex abundance. Initial values were taken from literature and PK/PD software facilitated for parameter estimation. Eventually, dosages and timing thereof were reported (initial dose = 44.8 mg, drug administered anew after 23 days at a dose of 40.3 mg). Mind, that for this estimation, the peripheral compartment mentioned in the model is not regarded (Schropp et al. 2019). Note, that each binding step has its own association rate. The first antigen is bond by a free antibody at rate k_{on}^{Ag1} while a dimer of the antibody and the second antigen undergo tetramer formation at rate $k_{on}^{Ag1}{}_{Ag2}$. While not explicitly calling for avidity, the association rate is regarded different after the antibody engaged in binding with another epitope. This is on the same line as previous models suggesting a factor altering secondary target binding affinity.

Table 3 Models for dynamics of bivalent bispecific antibodies and their same-cell targets

Reference	Model	Explanation
(Rhoden et al. 2016)	$\frac{d[Ab]}{dt} = -k_{on}^{Ag1}[Ag1][Ab] - k_{on}^{Ag2}[Ag2][Ab] + k_{off}^{Ag1}[AbAg1] + k_{off}^{Ag2}[AbAg2]$ $\frac{d[Ag1]}{dt} = -(k_{on}^{Ag1}[Ag1][Ab] + x_{Ag1}[Ag1][AbAg2]\frac{[Ag1]}{[Ag1_0]}) + k_{off}^{Ag1}([AbAg1] + [AbAg1Ag2])$ $\frac{d[Ag2]}{dt} = -k_{on}^{Ag2}([Ag2][Ab] + x_{Ag2}[Ag2][AbAg1]\frac{[Ag2]}{[Ag2_0]}) + k_{off}^{Ag2}([AbAg2] + [AbAg1Ag2])$ $\frac{d[AbAg1]}{dt} = k_{on}^{Ag1}[Ag1][Ab] - k_{off}^{Ag1}[AbAg1] + k_{off}^{Ag2}[AbAg1Ag2] - k_{on}^{Ag2}x_{Ag2}[Ag2][AbAg1]\frac{[Ag2]}{[Ag2_0]}$ $\frac{d[AbAg2]}{dt} = k_{on}^{Ag2}[Ag2][Ab] - k_{off}^{Ag2}[AbAg2] + k_{off}^{Ag1}[AbAg1Ag2] - k_{on}^{Ag1}x_{Ag1}[Ag1][AbAg2]\frac{[Ag1]}{[Ag1_0]}$ $\frac{d[AbAg1Ag2]}{dt} = k_{on}^{Ag1}x_{Ag1}[Ag1][AbAg2]\frac{[Ag1]}{[Ag1_0]} + k_{on}^{Ag2}x_{Ag2}[Ag2][AbAg1]\frac{[Ag2]}{[Ag2_0]} - (k_{off}^{Ag1} + k_{off}^{Ag2})[AbAg1Ag2]$	Antibody and antigen concentration are assumed evenly distributed within the medium. Initial binding of the antibody to its first antigen results in a hemisphere with the dimer at its center. The resulting change of antibody and second antigen concentration upon binding changes the likelihood of binding to the second target. Avidity is accounted for by a change in association rate. This change represents the effective concentration of the antigen to be bound and thus differs per antigen.
(Sengers et al. 2016)	$\frac{d[AbAg1]}{dt} = k_{on}^{Ag1}[Ab][Ag1] - k_{off}^{Ag1}[AbAg1] - k_{on}^{Ag2}x[AbAg1][Ag2] + k_{off}^{Ag2}[AbAg1Ag2]$ $\frac{d[AbAg2]}{dt} = k_{on}^{Ag2}[Ab][Ag2] - k_{off}^{Ag2}[AbAg2] - k_{on}^{Ag1}x[AbAg2][Ag1] + k_{off}^{Ag1}[AbAg1Ag2]$ $\frac{d[AbAg1Ag2]}{dt} = k_{on}^{Ag1}x[AbAg2][Ag1] + k_{on}^{Ag2}x[AbAg1][Ag2] - (k_{off}^{Ag1} + k_{off}^{Ag1})[AbAg1Ag2]$	The concentrations of targets are calculated as difference between total and bound antigens at time t. The antibody concentration is assumed constant and even. Avidity is accounted for by a change in association rate modelled by x.

(Vauquelin and Charlton 2013)	$\frac{d[Ab]}{dt} = k_{off}^{Ag1}[AbAg1] + k_{off}^{Ag2}[AbAg2] + (k_{off}^{Ag1} + k_{off}^{Ag2})[AbAg1\|Ag2Ab] - k_{on}^{Ag1}([Ab][Ag1] + [AbAg2][Ag1][Ab]) - k_{on}^{Ag2}([Ab][Ag2] + [AbAg1][Ag2][Ab])$ $\frac{d[Ag1]}{[dt]} = -k_{on}^{Ag1}[Ab][Ag1] + k_{off}^{Ag1}([AbAg1] + [AbAg1\|Ag2Ab] + [AbAg1Ag2])$ $\frac{d[Ag2]}{[dt]} = -k_{on}^{Ag2}[Ab][Ag2] + k_{off}^{Ag2}([AbAg2] + [AbAg1\|Ag2Ab] + [AbAg1Ag2])$ $\frac{d[AbAg1]}{[dt]} = k_{on}^{Ag1}[Ab][Ag1] - k_{off}^{Ag1}[AbAg1] + k_{off}^{Ag2}([AbAg1\|Ag2Ab] + [AbAg1Ag2]) - k_{on}^{Ag2}([AbAg1][Ag2][Ab] + x\,[AbAg1][Ag2])$ $\frac{d[AbAg2]}{[dt]} = k_{on}^{Ag2}[Ab][Ag2] - k_{off}^{Ag2}[AbAg2] + k_{off}^{Ag1}([AbAg1\|Ag2Ab] + [AbAg1Ag2]) - k_{on}^{Ag1}([AbAg2][Ag1][Ab] + x\,[AbAg2][Ag1])$ $\frac{d[AbAg1Ag2]}{[dt]} = k_{on}^{Ag1}x\,[AbAg2][Ag1] + k_{on}^{Ag2}x\,[AbAg1][Ag2] - (k_{off}^{Ag2} + k_{off}^{Ag1})[AbAg1Ag2]$ $\frac{d[AbAg1\|Ag2Ab]}{[dt]} = k_{on}^{Ag1}[Ag2Ab][Ag1][Ab] + k_{on}^{Ag2}[Ag1Ab][Ag2][Ab] - (k_{off}^{Ag1} + k_{off}^{Ag2})[AbAg1\|Ag2Ab]$	This model introduces competition between antibodies. [AbAg1|Ag2Ab] denotes a pair of target antigens that is bound by two individual antibodies. In this case, for both targets (e.g. on the cell surface) to be bound, no steric hindrance needs to be overcome. Assuming that the targets are not bound or unbound simultaneously. Binding of second binding arm when bound already is sterically hindered and the decrease in association constant is modelled with x.
Adjusted from (Vauquelin et al. 2014)	$\frac{d[Ab]}{dt} = k_{off}^{Ag1}[AbAg1] - k_{on}^{Ag1}[Ag1][Ab] + k_{off}^{c}[cAg1] - k_{on}^{c}[c][Ag1] + k_{off}^{Ag2}[AbAg2] - k_{on}^{Ag2}[Ag2][Ab]$ $\frac{d[Ag1]}{dt} = k_{off}^{Ag1}\left([AbAg1] + \frac{1}{\alpha'}[AbAg1Ag2]\right) - -k_{on}^{Ag1}[Ag1][Ab] + k_{off}^{c}([cAg1] + \frac{1}{\alpha_c{}'}[AbAg2\|cAg1]) - k_{on}^{c}[c][Ag1]k_{on}^{Ag1}\alpha\,[AbAg2][L] - k_{on}^{c}\alpha_c[AbAg2][Ag1][c]$ $\frac{d[Ag2]}{dt} = k_{off}^{Ag2}([AbAg2] + \frac{1}{\alpha'}[AbAg1Ag2]) - k_{on}^{Ag2}[Ag2][Ab] - k_{on}^{Ag2}\alpha\,[AbAg1][L]$ $\frac{d[c]}{dt} = k_{off}^{c}([cAg1] + \frac{1}{\alpha_c{}'}[AbAg2\|cAg1]) - k_{on}^{c}([c][Ag1] + \alpha_c\,[c][Ag1][AbAg2])$ $\frac{d[AbAg1]}{dt} = k_{on}^{Ag1}[Ag1][Ab] - k_{off}^{Ag1}[AbAg1] - k_{on}^{Ag2}\alpha\,[AbAg1][L] + \frac{k_{off}^{Ag2}}{\alpha'}[AbAg1Ag2]$ $\frac{d[AbAg2]}{dt} = k_{on}^{Ag2}[Ag2][Ab] - k_{off}^{Ag2}[AbAg2] - k_{on}^{Ag1}\alpha\,[AbAg2][L] + \frac{k_{off}^{Ag1}}{\alpha'}[AbAg1Ag2] - k_{on}^{c}\alpha_c\,[AbAg2][c][Ag1] + \frac{k_{off}^{c}}{\alpha_c{}'}[AbAg2\|cAg1]$ $\frac{d[cAg1]}{dt} = k_{on}^{c}[c][Ag1] - k_{off}^{c}[cAg1]$	An external molecule (c) competes for binding to the first antigen ($Ag1$) The model is adjusted in the sense that the targets are regarded one in the original publication. Here, they are taken apart. Mind however, that they are still assumed to be on the same cell, as [L] accounts for the concentration of the second target within the hemisphere surrounding the single bound antibody to the first target. The original publication does furthermore not contain an ODE for the bispecific antibody itself and is here thus shown as derives from the paper. Similarly, the dynamics of c are derived from the paper. $k_c\,\alpha_c[AbAg2][Ag1][c]$ accounts for binding of $Ag1$ which is in proximity of $Ag2$which is already bound to Ab. Confirmational changes upon $Ag2Ab$binding influences binding of $Ag1$to c in proximity of dimer. The same holds

	$\frac{d[AbAg2][cAg1]}{dt} = k_{on}^{Ag2}\ \alpha_c\ [cAg1][Ab][Ag2] - \frac{k_{off}^{Ag2}}{\alpha_c{}'}\ [AbAg2\|cAg1] - \frac{k_{off}^{c}}{\alpha_c{}'}[AbAg2\|cAg1] + k_{on}^{c}\ \alpha_c\ [c][Ag1][AbAg2]$ $\frac{d[AbAg1Ag2]}{dt} = k_{on}^{Ag2}\ \alpha\ [AbAg1][L] - \frac{k_{off}^{Ag2}}{\alpha'}\ [AbAg1Ag2] + k_{on}^{Ag1}\ \alpha\ [AbAg2][L] - \frac{k_{off}^{Ag1}}{\alpha'}\ [AbAg1Ag2]$	for $-k_c\ \alpha_c\ [AbAg2][c][Ag1] + \frac{k_{-c}}{\alpha_c{}'}\ [AbAg2][cAg1]$ for c binding $Ag1$ in proximity of an $AbAg2$ dimer
(Schropp et al. 2019)	$\frac{d[Ab]}{dt} = k_{off}^{Ag1}[AbAg1] + k_{off}^{Ag2}\ [AbAg2] - k_{on}^{Ag1}[Ab][Ag1] - k_{on}^{Ag2}[Ab][Ag2] + \frac{In_{IV}(t)}{V} + \frac{k_a\ AP}{V} + k_{-p}\frac{AP}{V} - k_{el}\ [\mathrm{Ab}]$ $\frac{d[Ag1]}{[dt]} = -k_{on}^{Ag1}[Ab][Ag1] + k_{off}^{Ag1}[AbAg1] - k_{on}^{Ag1}{}_{Ag2}[\mathrm{AbAg2}][\mathrm{Ag1}] + k_{off}^{Ag1}{}_{Ag2}[\mathrm{AbAg1Ag2}] + k_{syn_{Ag1}} + k_{deg_{Ag1}}[\mathrm{Ag1}]$ $\frac{d[Ag2]}{[dt]} = -k_{on}^{Ag2}[Ab][Ag2] + k_{off}^{Ag2}\ [AbAg2] - k_{on}^{Ag2}{}_{Ag1}[\mathrm{AbAg1}][\mathrm{Ag2}] + k_{-3}[\mathrm{AbAg1Ag2}] + k_{syn_{Ag2}} + k_{deg_{Ag2}}[\mathrm{Ag2}]$ $\frac{d[AbAg1]}{[dt]} = k_{on}^{Ag1}[Ab][Ag1] - \left(k_{off}^{Ag1} + k_{int_{AbAg1}}\right)[AbAg1] - k_{on}^{Ag2}{}_{Ag1}[AbAg1][Ag2] + k_{off}^{Ag2}{}_{Ag1}[AbAg1Ag2]$ $\frac{d[AbAg2]}{[dt]} = k_{on}^{Ag2}[Ab][Ag2] - \left(k_{off}^{Ag2} + k_{int_{AbAg2}}\right)[AbAg2] - k_{on}^{Ag1}{}_{Ag2}[AbAg2][Ag1] + k_{off}^{Ag1}{}_{Ag2}[AbAg1Ag2]$ $\frac{d[AbAg1Ag2]}{[dt]} = k_{on}^{Ag1}{}_{Ag2}\ [AbAg2][Ag1] + k_{on}^{Ag2}{}_{Ag1}\ [AbAg1][Ag2] - \left(k_{off}^{Ag2}{}_{Ag1} + k_{off}^{Ag1}{}_{Ag2} + k_{int_{AbAg1Ag2}}\right)\ [AbAg1Ag2]$ Drug availability: Central compartment: $\frac{d\ AD}{dt} = In_{sc}(f,t) - k_a AD$ Peripheral compartment: $\frac{d\ AP}{dt} = k_p\ V\ [Ab] - k_{-p}\ AP$	This model allows for biosynthesis of the epitopes, their degradation, internalization of dimers and tetramers and distribution of the drug into a central and peripheral compartment.

T cell engaging bispecific antibodies

As mentioned previously, binding events of T-BsAbs happen independently (Sengers et al. 2016). However, avidity can here be regarded as a phenomenon resulting from different affinities of antibody arms ultimately influencing the target involvement and delocalization of the administered antibody (Slaga et al. 2018). The dynamics of complexes formed between the antibody and its two soluble targets can be modelled. Suitable models may help in finding optimum antibody constructions. Adverse effects of T-BsAbs (often cytokine release syndrome) may be minimized by following certain dosing regimes. Quantifying and identifying patterns in cytokine response upon different dosages, duration- and frequency of drug administration may help to find an optimum therapy plan (Chen et al. 2019).

We consider antibodies that are active, thus bringing about the cytotoxic mechanisms of T cells, when both the tumor associated antigen (TAA) is covalently bound on one arm and the T cell on the other (e.g., through CD3). The abundance of such a tetramer is then determined by the availability of the administered drug, by the total concentrations of the target as well as by the binding affinities to both targets. Assuming steady-state of the T-cell population, one finds the concentration of tetramers to be proportional to the product of the drug concentration and the TAA concentration (Chen et al. 2019).

When assuming that binding affinities do not change upon dimer formation, one finds the concentration of tetramer to follow (Chen et al. 2019):

$$[AbAg1Ag2] = \frac{[Ab][Ag]_{Tumor}[Ag]_{T-cell}}{k_1 k_2}$$

With $[Ag]_{Tumor}$ and $[Ag]_{T-cell}$ being targets of the bispecific antibody and k_1 and k_2 the binding affinity of the antibody to the respective targets (Chen et al. 2019). We thus find that although binding affinities are unaffected in T-BsAbs, the product of affinities to individual targets does determine the abundance of tetramer complexes. The model was further developed to include cytokine release. It was used to explain adverse effects after administration by quantifying increased cytokine release. Furthermore, dose escalation as compared to a single higher dose administration was shown to be associated with higher cytokine release, supporting current therapy ideas of "priming" strategies (Chen et al. 2019).

C. Conclusion

While masked in different models, avidity in cis-binding antibodies comes down to a single concept. It is the phenomenon of change in binding dynamics as a result of dimer formation. This brings about changes in microenvironment of the antibody, thereby in concentration of antigen in the proximity of the cell-bound antibody. This can be modelled using the average distance between the two antibody arms on the cell surface, leading to the actual antigen concentration in the sphere surrounding the dimer (Crothers and Metzger 1972; Perelson et al. 1980; Kaufman and Jain 1992). Confirmational changes of cell-bound antigens upon antibody binding may also play a role in changes of secondary binding affinity (Vauquelin et al. 2014). The effect of primary binding on secondary binding may thus be broken down into (a) changes in association constant and thus binding affinity (whether alike for both targets (Harms et al. 2012; Sengers et al. 2016) or different (Rhoden et al. 2016)) and (b) changes in local concentrations of antibody and antigen. Of course, these concepts are not independent. It may also be regarded as a single avidity factor (x) as introduced by (Harms et al. 2012) (as depicted in Figure 1). For the sake of insights into the relevance of different aspects, one may however want to regard them separately.

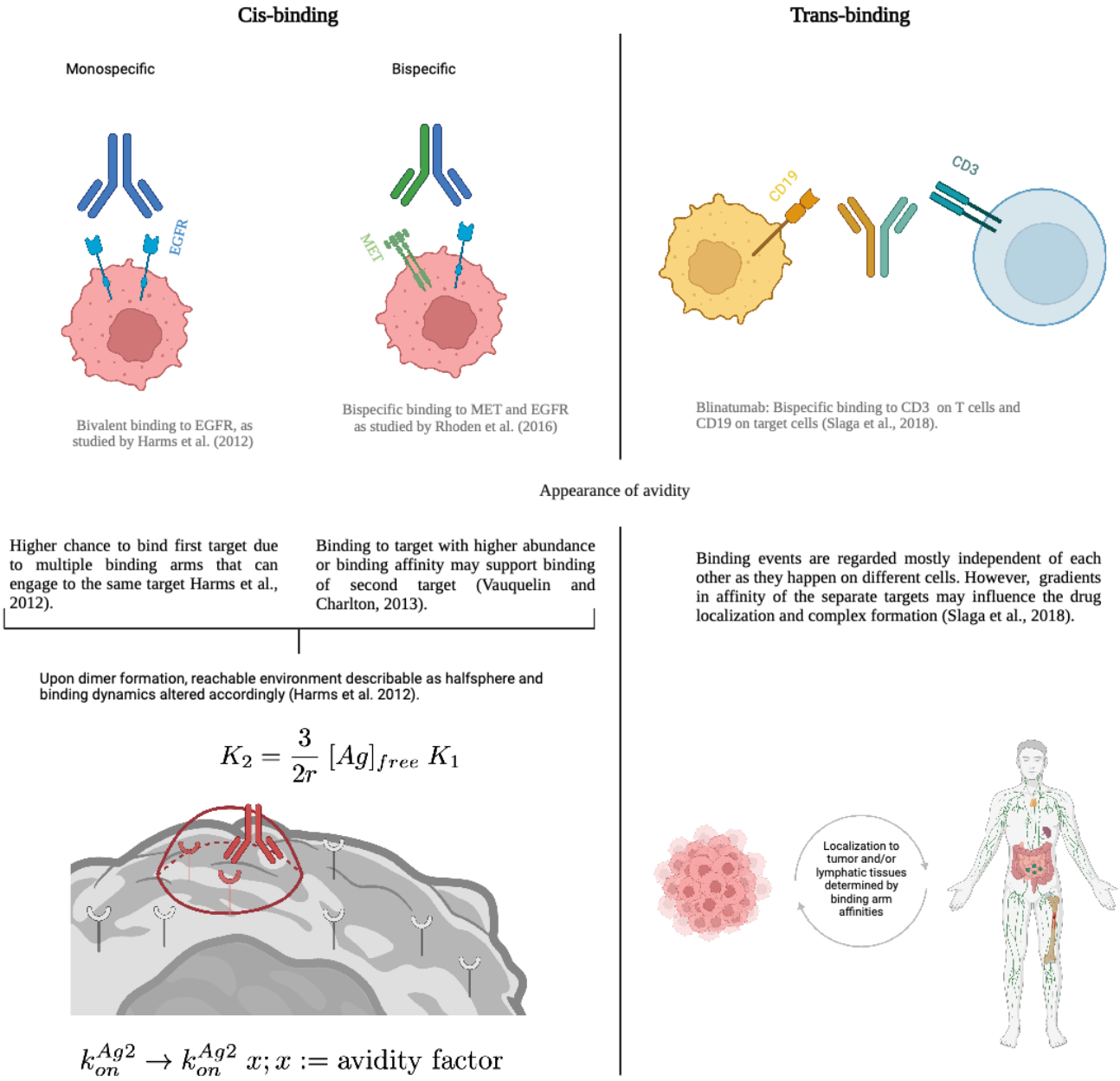

Figure 1 Avidity in cis- and trans-binding bivalent (bispecific) antibodies. As cis-binding antibodies bind their first target, the environment of the dimer can be modelled as a hemisphere in which the antibody can rotate and find a second target. Following Perelson et al. (1981), the equilibrium constant of the second binding can be modelled using the free epitope concentration and the reach of the antibody. On a population level, multiple models call for a change in binding affinity to the second target due to changes in the local environment of the bound antibody (Harms et al. 2012, 2014; Rhoden et al. 2016; Sengers et al. 2016). This concept is here summarized using an avidity factor x (Table 2, Table 3). Competition between antibodies, with an external molecule and drug distribution over several compartments can be added in more complex models (see Table 3, (Vauquelin and Charlton 2013; Vauquelin et al. 2014; Schropp et al. 2019), reference stated respectively). Trans-binding antibodies binding arms do not pose changes in binding affinity onto each other (Sengers et al. 2016). The combinations of affinity of the binding arms however introduce an effect on the localization of the drug (Bortoletto et al. 2002; Mandikian et al. 2018). As this reflects an effect in tumor targeting not equaling the sum of effects of the binding arms, this phenomenon is here also considered as avidity. Created with BioRender.com.

Remembering that affinity is a Chemomeasure (Agoram and Graaf 2012), avidity rather reflects a concept depending both on the drug itself (e.g., affinities of the various binding arms) and on systemic circumstances (e.g., distance of the targets). While this may sound as a contradiction to avidity introduced by Harms et al. (2014) as being characteristic of an antibody, this is not necessarily the case. Seeing avidity as the net-effect of above-described phenomena, the tendency of an antibody to establish a second binding is only one of many aspects. Such may indeed remain constant for a certain antibody, supported by modeling of second binding equilibrium as a function of antibody reach and epitope concentration by Crothers and Metzger (1972) (Table 1). The resulting complex formations and binding strengths however may eventually differ depending on the system. Note, that the antibodies' tendency to engage in second binding loses relevance as epitope concentration increases, favoring dimer- over tetramer formation (Harms et al. 2014).

While some models introduce an avidity factor that is alike for both antigens of a bivalent antibody (Harms et al. 2012; Vauquelin and Charlton 2013; Sengers et al. 2016), other allow different effects per antibody or include their concentration surrounding the dimer (Vauquelin et al. 2014; Rhoden et al. 2016; Schropp et al. 2019). Diving into more detailed models, one may want to include intrapopulation competition of the antibodies (Vauquelin and Charlton 2013) or an external molecule binding to one of the antigens (Vauquelin et al. 2014). Considering that an administered drug in vivo likely distributes throughout the body, it is realistic to regard a model with multiple compartments. Furthermore, one may want to add the elimination of the administered antibody and synthesis and degradation of the cell-bound targets (Schropp et al. 2019). Eventually however, quasi-equilibrium assumptions and steady states of the antigens are required to allow for a realistic number of parameters to be estimated. When attempting to model antibody dynamics one thus needs to balance mechanisms and pathways to be considered and identifiability of parameters.

T-BsAbs undergo binding to targets on different cells. This leaves the binding affinities largely independent of one another. The interplay of different affinities may however affect the localization of an antibody (Slaga et al. 2018) and may in broader terms also be considered as avidity.

For future research, models as introduced here may help in the design of antibodies that provide the highest possible tetramer concentration at low drug concentrations. These quantifications may aid in minimizing side effects and spare healthy tissue. Of course, bivalent, and bispecific antibodies are only the tip of the iceberg. Multivalency and -specificity is in the focus of oncological research and was out of the scope of this report. However, quantifications of avidity as a function of multiple affinities, antigen concentrations and spatial circumstances may be regarded in future research.

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