Multiple Myeloma: Therapeutic delivery of antibodies and aptamers

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Abstract Multiple myeloma is the second most common haematological malignancy in adults, accounting for 2% of all cancer related deaths in the UK. Current chemotherapy-based regimes are insufficient, as most patients relapse and develop therapy resistance. This review focusses on current novel antibody- and aptamer-based therapies aiming to overcome current therapy limitations, as well as their respective limitations and areas of improvement. The use of computer modelling methods, as a tool to study and improve ligand-receptor alignments for the use of novel therapy development will also be discussed, as it has become a rapid, reliable and comparatively inexpensive method of investigation.

Keywords
Multiple myeloma, Antibody therapy, Aptamer therapy, Antibody-Drug conjugates, Aptamer-Drug conjugates, molecular docking, nanoparticles.

1: Multiple myeloma: Introduction to disease and current therapies

Epidemiology, Pathophysiology and Statistics:
Multiple myeloma (MM) is a haematological cancer which arises from abnormal plasma cells [1]. Globally it is the second most common haematological malignancy in adults after non-Hodgkin lymphoma, accounting for 10% of haematologic malignancies [2, 3]. MM cells are mainly localised in the bone marrow, although during the later stages of the disease they can infiltrate into the peripherial blood and extramedullary sites, such as the skeleton [4, 5].

MM is most commonly characterised by the secretion of a monoclonal immunoglobulin protein known as M protein or its free light kappa and lambda chains, which are released in approximately 97% of patients [6, 7]. Clinically, MM patients are diagnosed by the effects of M protein secretion in the body, which include renal insufficiency (due to the M protein light chains exhibiting nephrotoxicity), anaemia, hypercalcaemia, bone marrow plasma cell percentage ≥ 60%, serum free light chain ≥ 100 and bone disease with lytic lesions [8]. There is no specific genetic mutation that leads to the transformation of healthy cells into MM, as chromosomal translocations, aneuploidy, DNA methylation and microRNA expression have all been associated with an increased propensity for MM development [1]. Nearly all cases of MM are preceded by monoclonal gammopathy of undetermined significance (MGUS), which can then progress to smouldering MM (SMM), both of which are asymptomatic precursor states [9]. A study by Weiss *et al.* (2009) [10] demonstrated that at least 90% of MM patients suffer from pre-existing MGUS or SMM. However, the authors were not able to distinguish between these groups. MGUS has been found in 2.4% of the population over 50 years of age, with 0.5% to 1% of these individuals progressing to develop SMM per year [11]. It has also been described that annually 10% of patients with SMM will go on to develop MM [12].

It was estimated that in 2020, around 176,400 individuals globally suffered from MM, with 117,000 MM related deaths [13]. An International Agency for Research on Cancer (IARC) 2018 GLOBOCAN study concluded that in the UK, 1.9% of all new male cancers could be attributed to MM, as well as 2% of all cancer deaths. For UK females, 1.5% of all 2018 new cancer cases were attributed to MM, as well as 1.8% of all deaths. MM incidence is also seen to be 2-3 times higher in African individuals compared to Caucasian individuals, with lowest incidence rates observed in Asian and Hispanic populations [14, 15].

Treatment:
MM treatment strategies have significantly improved over the last 15 years with the introduction of proteosome inhibitors, immunomodulatory agents, and anti-CD38 antibodies. Currently, a multidrug approach is utilised based on patient co-morbidities and the ability to fulfil eligibility criteria for autologous stem cell transplantation (ASCT) [1]. In ASCT eligible patients, upfront intensive multidrug combinations are used based on national guidelines; including from the National Institute for Health and Care Excellence (NICE; England (UK)), Scottish Medicines Consortium (SMC; Scotland (UK)), and the National Comprehensive Cancer Network (NCCN; US), but also on international guidelines, such as the EHA-ESMO

clinical ractice guidelines [16] . Daratumumab, in combination with bortezomib, thalidomide and dexamethasone have most recently been approved for upfront therapy in those eligible for ASCT in the UK (NICE and SMC, 2021) [17]. Post-ASCT, maintenance therapy with lenalidomide has been shown to prolong progression free survival and overall survival [18, 19]. In non-ASCT eligible patients, a multidrug approach is used until disease progression [18, 19].

Patient treatment stratification is based on disease cytogenetics. MM can be divided into two primary cytogenetic abnormalities: trisomies (in chromosomes 3, 5, 7, 9, 11, 15 and 17) and translocations (e.g. t(11;14), t(4;14), t(6;14), t(14;16) and t(14;20)) involving the immunoglobulin heavy chain (IgH) gene [20, 21]. These abnormalities have been linked to different therapy responses and prognosis, with patients with trisomies having an overall better disease prognosis and better responses to lenalidomide-based therapy compared to patients with translocations. A study by Vu *et al.* (2015) [22] observed that 79% of MM patients with trisomies showed exceptional responses to lenalidomide-based therapy, with trisomies accounting for around 50% of all MM cases. Traditionally, patients with translocations have had inferior disease prognosis, however, they have been observed to respond to bortezomib-containing therapies and ASCT, achieving overall survival rates similar to patients with standard-risk MM [23].

Unfortunately, the majority of MM patients will relapse, with patients who go through various relapses showing minimal response rates to treatment regimens [24]. Relapse is due to the existence of chemoresistant MM cells, that exhibit protection against apoptosis induced by bortezomib and lenalidomide, and therefore continue to proliferate. Moreover, these chemotherapeutic agents are associated with particular toxicities, that can be detrimental to the patient, especially as the majority of MM sufferers are elderly patients [25]. These side effects include anaemia, risk of developing infections, bone pain, and bone loss among others. They arise due to chemotherapeutics non-selectively targeting all rapidly dividing cells in the body [25].

Another main cause of relapse is the presence of MM stem cells (MMSCs). These cells are CD138 negative (CD138$^-$), which promotes the activity of aldehyde dehydrogenase 1 (ALDH1), a marker for both normal haematopoietic stem cells (HSCs) and MMSCs. Furthermore, similar to HSCs, MMSCs possess self-renewal and chemoresistant properties, the latter being partly associated with reduced cycling/proliferation as these cells have exited the cell cycle, and are normally in G_0 phase [26].

In vivo studies by Reghunathan and colleagues (2013) [26] showed that xenografts from NOG mice implanted with CD138$^-$ MM cells lead to tumour initiation and disease progression in 100% of cases (6/6 xenografts). These cells were also capable of producing CD138$^+$ cells, which represent the majority of the malignant cells in MM. Reghunathan *et al.* also assessed the effects of implanting CD138$^+$ cells into NOG mice, and found that tumour initiation only occurred in 33% of cases (2/6 xenografts), with tumour development not being as extensive as in CD138$^-$ cells. This is due to the fact that terminally differentiated CD138$^+$ MM cells,

although form the majority of the tumour bulk, are unable to sustain clonogenic growth indefinitely, while CD138- cells can [26]. This highlights the need for new and safe therapies for MM that are capable of targeting both CD138+ (bulk MM cells) and CD138− cells (MMSCs).

Clinical trials are currently exploring the use of immune therapies in MM, such as those based on monoclonal antibodies (mAbs) [1]. However, more recently the use of aptamers is also being researched [27]. These two therapeutic approaches in MM are compared below to assess progress in the development of novel and effective MM therapies.

2: Alternative Approaches

Antibody therapy:

Antibody therapy has considerably revolutionised the field of clinical oncology as increased knowledge in key cellular pathways has led to the identification of the role of humoral immunity in cancer and the potential therapeutic use of antibodies [28]. Antibodies are glycoproteins that can specifically and effectively bind to different protein-based molecular structures, as well as nucleotides [29, 30]. There are nine sub-classes of antibodies in humans (IgG_{1-4}, IgM, IgE, IgA_{1-2}, and IgD), with IgG representing most of the available therapeutic antibodies (Irani *et al*., 2015) [31]. Several therapeutic antibodies, such as elotuzumab [32, 33], Isatuximab [34, 35] and daratumumab [36, 37], show promising results in improving progression free survival alone or in combination with other therapies in patients with newly diagnosed MM or relapsed/ refractory MM. Many antibodies are extensively being explored for the treatment of MM (Table 1).

Table 1: Antibodies against multiple myeloma.

Target antigen	Antibody	Developer	Source	Class	Development stage (ClinicalTrials.gov Identifier)	Reference
SLAMF7	Elotuzumab	Bristol-Myers Squibb and AbbVie	Humanised	IgG_1	Phase III (NCT01239797)	[38, 39] [32]
CD38	Isatuximab	ImmunoGen and Sanofi-Aventis	Chimeric (mouse/human)	IgG_1	Phase III (NCT02990338)	[34, 40]
CD38	Daratumumab	Genmab and Janssen Biotech	Human	IgG_1	Phase III (NCT02136134)	[41] [42] [36]
CD38	MOR202	MorphoSys	Human	IgG_1	Phase I/II (NCT01421186)	[43]
CD38	TAK-079	Takeda Oncology	Humanised	IgG_1	Phase I (NCT04017130)	[44]

CD20	Rituximab	IDEC Pharmaceuticals, Biogen, Genentech, and Hoffmann–La Roche	Chimeric (murine/human)	IgG$_1$	Phase II (NCT00258206)	[45]
CD40	Dacetuzumab	Seattle Genetics	Humanised	IgG$_1$	Phase I (NCT00079716)	[46]
CD56	Lorvotuzumab	ImmunoGen	Humanised	IgG$_1$	Phase I (NCT00991562)	[47]
CD70	SGN-70	Seattle Genetics	Humanised	IgG$_1$	Phase I (NCT02216890)	[48]
CD200	Samalizumab (ALXN6000)	Alexion Pharmaceuticals	Humanised	IgG$_{2,4}$	Phase I/II (NCT00648739)	[49]
CD317	XmAb5592	LeBow Institute for Myeloma Therapeutics	Humanised	IgG$_1$	Preclinical development	[50]
GM-2	BIW-8962	BioWa	Humanised	IgG$_1$	Phase I (NCT00775502)	[51]
IGF-1R	AVE1642	Sanofi Aventis	Humanised	IgG$_1$	Phase I (NCT01233895)	[52]
IGF-1R	Dalotuzumab	Merck & Co	Humanised	IgG$_1$	Phase I (NCT00701103)	[53]
IGF-1R	Figitumumab	Pfizer	Human	IgG$_2$	Phase I (NCT01536145)	[54]
KIR	IPH2101	Innate Pharma	Human	IgG$_4$	Phase I (NCT00552396)	[55]
KIR	Lirilumab	BMS	Human	IgG$_4$	Phase I/II (NCT01592370)	[56]
HLA-DR	1D09C3	GPC Biotech AG and MorphoSys	Human	IgG$_4$	Preclinical development	[57]
TRAILR1 (DR4)	Mapatumumab	AstraZeneca (Cambridge Antibody Technology), and GlaxoSmithKline	Human	IgG$_1$	Phase II (NCT00315757)	[58]
IL-6	Siltuximab	Janssen Biotech	Chimeric (mouse/human)	IgG$_1$	Phase II (NCT01484275)	[59]
IL-6	Elsilimomab	Opi	Murine	IgG$_1$	Preclinical development	[60]
IL-6 receptor	Tocilizumab	Chugai and Roche	Humanised	IgG$_1$	Phase I (NCT04910568)	[61]
VEGF	Bevacizumab	Novartis and Roche	Humanised	IgG$_1$	Phase II (NCT00473590)	[62]
FGFR3	PRO-001	University of Toronto and ProChon Biotech	Human	IgG$_1$	Preclinical development	[63]
FGFR3	MFGR1877S	Genentech	Human	IgG$_1$	Phase I (NCT01122875)	[64]
RANKL	Denosumab	Amgen	Human	IgG$_2$	Phase II (NCT03839459)	[65]
DKK1	BHQ880	Novartis	Humanised	IgG$_1$	Phase II (NCT01302886)	[66]

Activin A	ACE-011	Celgene and Acceleron	Human	IgG$_1$	Phase I (NCT01562405)	[67]
ICAM-1 (CD54)	BI-505	BioInvent International AB	Human	IgG$_1$	Phase I (NCT01025206)	[68]
APRIL/BAFF	Tabalumab	Eli Lilly	Human	IgG$_4$	Phase II (NCT01602224)	[69]
APRIL/BAFF	BION-1301	Aduro Biotech	Humanised	IgG$_4$	Phase I/II (NCT03340883)	[70]
CXCR4	Ulocuplumab	BMS	Human	IgG$_1$	Phase I (NCT01359657)	[71]
CD137	Urelumab	BMS	Human	IgG$_4$	Phase I (NCT02252263)	[72]
Sclerostin	Romosozumab	UCB	Humanised	IgG$_2$	Preclinical development	[73] [74]
PD-1	Cemiplimab	Sanofi	Human	IgG$_4$	Phase I/II (NCT03194867)	
PD-1	Nivolumab	Medarex, Ono Pharmaceutical, and Bristol-Myers Squibb	Human	IgG$_4$	Phase I/II (NCT03292263)	[75]
PD-L1	Durvalumab	Medimmune/AstraZeneca	Human	IgG$_1$	Phase II (NCT03000452)	[76]
PD-L1	Atezolizumab	Genentech/Roche	Humanised	IgG$_1$	Phase I/II (NCT03312530)	[76]
EGFR	Cetuximab	Eli Lilly and Merck KGaA.	Chimeric (mouse/human)	IgG$_1$	Phase II (NCT01524978)	[77]

Antibodies have also been used to deliver cytotoxic drugs and nanoparticles (NPs) to cancer cells. Antibody-Drug Conjugates (ADCs) use the specificity of the antibody towards any specified target, and the cell killing capacityof the cytotoxic agent that has been chemically conjugated to the antibody (Nejadmoghaddam *et al*., 2019) [78]. This enables the antibodies to act as "Trojan horses", that can deliver cytotoxic molecules specifically into cancer cells.

The mechanism of action and optimisation of various ADCs have been comprehensively reviewed by Birrer *et al*. (2019) [79]. In 2020, the Food and Drug Administration (FDA) granted accelerated approval for belantamab mafodotin (Blenrep, GlaxoSmithKline) to be used for adult patients with relapsed or refractory MM who had received at least four prior therapies, including an anti-CD38 monoclonal antibody, a proteasome inhibitor, and an immunomodulatory agent (FDA, 2020). Table 2 describes the components of different ADCs that are currently under-development for the treatment of MM.

Table 2: ADCs against multiple myeloma

Target antigen	ADC	Developer	Source	Isotype	Conjugate	Development stage (ClinicalTrials.gov Identifier)	Reference
CD138	Indatuximab Ravtansine (BT062)	ImmunoGen	Chimeric (murine/human	IgG$_4$	DM1 maytansinoid	Phase I/II (NCT01638936)	[80]
CD74	Milatuzumab	Immunomedics	Humanised	IgG$_1$	Doxorubicin	Phase I/II (NCT01101594)	[81]
CD74	STRO-001	Sutro Biopharma	Aglycosylated human	IgG$_1$	Para-azidomethyl-l-phenylalanine (pAMF)	Phase I (NCT03424603)	[82]
CD20	Yttrium-90 ibritumomab	Biogen Idec	Murine	IgG$_1$	Radioconjugate	Phase II (NCT01207765)	[83]
CD20	Iodine-131 tositumomab	Corixa (GlaxoSmithKline)	Murine	IgG$_{2a}$	Radioconjugate	Phase II (NCT00135200)	[84]
CD56	Lorvotuzumab mertansine (IMGN901)	ImmunoGen	Humanised	IgG$_1$	DM1 maytansinoid	Phase I (NCT00346255)	[85]
CD38	TAK-573	Takeda Oncology	Human	IgG$_4$	Interferon alpha (IFN-α)	Phase I/II (NCT03215030)	[86]
SLAMF7	Azintuxizumab vedotin (ABBV-838)	AbbVie	Humanised	IgG$_1$	Monomethyl auristatin E (MMAE)	Preclinical development	[87]
SLAMF2	SGN-CD48A	Seattle Genetics	Humanised	-	MMAE	Phase I (NCT03379584)	[88]
SLAMF6	SGN-352A	Seattle Genetics	Humanised	-	Pyrrolobenzodiazepine (PBD) dimers	Phase I (NCT02954796)	[88]
IL-15	ALT-803	Altor BioScience	IgG$_1$Fc	IgG$_1$	Mutated Interleukin-15 (IL-15) (N72D) linked to the IL-15R sushi domain	Phase I (NCT02099539)	[89]
BCMA	Belantamab Mafodotin	GlaxoSmithKline	Humanised afucosylated	IgG$_1$	Monomethyl auristatin F	Approved	[90]
BCMA	MEDI2228	MedImmune	Humanised		PBD	Phase I (NCT03489525)	[91]
BCMA	AMG 224	Amgen	-	IgG$_1$	DM1 maytansinoid	Phase I (NCT02561962)	[92]
BCMA	HDP-101	Heidelberg Pharma	-	-	Amanitin	Phase I/II (NCT04879043)	[93]
CD47	TTI-621	Trillium Therapeutics	Linking the N-terminal CD47 binding domain of human SIRPα with	IgG$_1$ Fc	Signal regulatory protein α (SIRPα) binding domain	Preclinical development	[94]

Target antigen		Developer	Source			Development stage (ClinicalTrials.gov Identifier)	Reference
			the Fc domain of human IgG_1				
CD47	TTI-622	Trillium Therapeutics	Linking the N-terminal CD47 binding domain of human SIRPα with the Fc domain of human IgG4	IgG_4 Fc	SIRPα binding domain	Preclinical development	[94]
CD33	Lintuzumab-actinium 225	Seattle Genetics	Humanised	IgG_1	Actinium 225	Phase I (NCT02998047)	[95]
FcRH5	DFRF4539A	Genentech	Humanised	IgG_1	MMAE	Phase I (NCT01432353)	[96]

Multi-specific antibodies, which can target different biomarkers simultaneously, have been exploited for the treatment of MM, as summarised in Table 3. The multi-specific approach is based on technologies such as Amgen's bispecific T cell engagers (BiTEs). BiTEs are recombinant bispecific proteins composed of two linked scFvs from two different antibodies, one targeting the antigens on the surface of malignant cells and the other targeting cell-surface molecule on T cells (such as CD3ε) [97]. Another approach was developed by Genmab, the DuoBody platform, with the aim of creating bispecific antibodies that can bind to two different epitopes, either on the same or on different targets [98, 99]. These technologies can enhance the overall efficacy by targeting multiple targets and generating enhanced anti-tumour activity.

Table 3: Multi-specific antibodies against multiple myeloma

Target antigen	Multi-specific antibodies	Developer	Source	Development stage (ClinicalTrials.gov Identifier)	Reference
CD3-BCMA	Pacanalotamab (BI-836909)	Amgen	BiTEs technology	Phase I (NCT02514239)	[100]
CD3-BCMA	EM801	EngMab AG Celgene	Asymmetric two-arm IgG_1-based human antibody with two binding sites for BCMA and 1 binding site for CD3	Preclinical development	[101]
CD3-BCMA	Teclistamab (JNJ-64007957)	Genmab, Janssen	DuoBody technology	Phase I (NCT04696809)	[98]
CD3-BCMA	Elranatamab (PF-06863135)	Pfizer	A humanised IgG CD3 bispecific mAb that utilizes anti-BCMA and anti-CD3 targeting arms that are paired through hinge-mutation technology within an IgG_{2a} backbone.	Phase I (NCT03269136)	[102]
CD3-BCMA	BCMA-TCB2/EM901	Celgene	Two-arm IgG_1-based human antibody	Phase I (NCT03486067)	[103]
CD3-BCMA	TNB383B/TNB-384B	TeneoBio	Two anti-BCMA heavy chain variable domains linked to a unique anti-CD3 T-cell recruiting arm	Phase I (NCT03933735)	[104]
CD16A (NK cells)-BCMA	AFM26	Affimed	Tetravalent, bispecific antibody targeting BCMA and CD16A (FcγRIIIa) to selectively redirect natural killer (NK-)cell lysis	Preclinical development	[105]

CD3-GPRC5D	Talquetamab (JNJ-64407564)	Genmab and Janssen	DuoBody technology	Phase I (NCT04773522)	[99]
CD3-FcRH5	Cevostamab (BFCR4350A)	Genentech	BiTEs technology	Phase I (NCT03275103)	[106]
CD3-CD138	h-STL002, m-STL002	Abgent (Suzhou) Biotechnology AND Persongen Biomedicine	ScFvs cloned from two hybridoma cells were combined with anti-CD3 OKT-3 ScFv to generate two recombinant bispecific antibodies	Preclinical development	[107] [108]
CD3-CD138	STL001	The Cyrus Tang Hematology Center	CD138-ScFv-OKT3-ScFv-pFUSE-hIgFc	Preclinical development	[107]
NKG2D-CS1	CS1-NKG2D	The Ohio State University	Fusing an anti-CS1 single-chain variable fragment (scFv) and an anti-NKG2D scFv (CS1-NKG2D biAb).	Phase I (NCT02203825)	[109]
BCMA-BCMA	BiFab-BCMA	California Institute for Biomedical Research	Using a modular semisynthetic method in which two antigen binding fragments (Fabs) are site-specifically conjugated via unnatural amino acids	Preclinical development	[110]
CS1-CS1	BiFab-CS1	California Institute for Biomedical Research	Using a modular semisynthetic method in which two antigen binding fragments (Fabs) are site-specifically conjugated via unnatural amino acids	Preclinical development	[110]
CD16A-BCMA-CD200	Trispecific antibody (TriFlex)	Affimed	Fv domains specific for BCMA and CD200 isolated from human naïve human antibody libraries by phage display and fused to the N- and C-terminus of the anti-CD16A VL-containing chain	Preclinical development	[105]

Aptamer Therapy:

Aptamers are synthetic single-stranded oligonucleotides that bind to a variety of targets with high affinity and specificity due to their complex tertiary structure, and have been recognised as potential cancer therapy candidates [111]. Due to their ability to differentiate between targets, aptamers are thought of as 'smart ligands 'and have been termed 'nucleic acid antibodies [112] as well as 'chemical antibodies' [27]. Pegaptanib sodium (Macugen®) is the only aptamer that has been approved by the FDA since 2004, which targets the vascular endothelial factor (VEGF-165) isoform to treat age-related macular degeneration [113]. In terms of cancer therapy, there are over 40 aptamers currently in development that have been proven to be effective *in vivo* against cancer models, with two aptamers, AS1411 and NOX-A12, successfully reaching clinical trials [111, 114]; NOX -A12 has been tested in a phase II study, in combination with bortezomib and dexamethasone for the treatment of relapsed MM (NCT01521533)[114]. Aptamers are produced via the systematic evolution of ligands by exponential enrichment (SELEX) technology [115]. A detailed description of the production process is described by Fu and Xiang (2020) [116].

In cancer therapy, aptamers can be used as free molecules to target specific cancer biomarkers acting as agonists or antagonists. So far, both AS1411 and NOX-A12 that have reached clinical trials are antagonistic aptamers, targeting nucleolin [117] and CXCL12 [118], respectively. The development of more complex forms of aptamer-based therapies include,

among others, bispecific aptamers and aptamer-antibody complexes (oligobodies) [119, 120]. Furthermore, as with antibodies, aptamers can be endocytosed through ligand binding with specific cell membrane receptors. This can be exploited in targeted drug delivery where aptamers conjugated to therapeutic agents can selectively deliver their payload into target cancer cells without affecting healthy ones.

As a result, aptamers can be conjugated (covalently or non-covalently) to drugs forming aptamer-drug conjugates (ApDCs), therapeutic oligonucleotides (siRNAs, miRNAs and shRNAs) forming aptamer-oligonucleotide conjugates (ApOCs) and nanocarriers [116, 121]. The strategies by which aptamers are modified for therapeutic use has been reviewed by Adachi and Nakamura (2019) [122]. In order to test the binding ability of antibodies and aptamers, the use of computer modelling has been used as a predictor of potential therapeutical targets. An overview of the uses, methodology, advantages and disadvantages of molecular docking is described in the next section.

3: Computational Drug Design

Detailed interactions between peptides and aptamers or antibodies can be investigated using computational modelling methods, as proposed in the early 1980s to study ligand-receptor possible complexes through molecular docking [123]. The docking procedure examines feasible alignments of ligand and receptor, and evaluates them in terms of steric overlap [123]. For each possible ligand and receptor complex, the software calculates the potential energy, which reflects the quality of the match; biologically active complexes are one of the low (but not necessarily lowest) energy configurations. Obtained complexes of the lowest energy (the best score) are considered as the most likely candidates to be used in further investigations towards drug design, as well as understanding of signal transduction processes, where the associations between biologically relevant molecules play a central role. Docking might therefore explain in detail the fundamentals of the biochemical processes of an aptamer or antibody binding to its target. Moreover, the method might be used to predict the most plausible binding conformation, and suggest the route for improvement of binding by, for example, point mutations on certain nucleic acids or amino acids of either the aptamer/ antibody or the target molecule, respectively.

The advantages of docking in cases, such as, the virtual screening of a library of potential drug candidates or in investigations of ligand binding mode, has led to the wide usage of the method. Nowadays, antibody and aptamer ligands are fully flexible [124], and although still computationally expensive, it is possible to include target flexibility. Such a method gives more accurate description of the binding site, but neglects the possibility of receptor rearrangement upon ligand binding. This method, usually called standard docking, might be then described as reasonably fast (e.g., computationally inexpensive) and is extensively used in rough screening of thousands of ligands [125] relevant for the pharmaceutical industry. The predictions produced by such screening need to be further investigated and validated, ideally by other, more advanced methods, such as for example Molecular Dynamics (MD). The docking procedure predicts and proposes several starting structures, while MD reports some additional details on dynamic complex behaviour. Due to initial usage of prediction methods (docking and MD), laboratory costs and time required for validation is substantially reduced.

In contrast to the above method that combines standard docking with usually multiple MD simulations, one might consider using dynamic docking which is more accurate, but more computationally expensive. Dynamic docking allows the prediction of the docked geometry of the ligand-receptor complex without any initial assumptions, obtaining detailed description of the association path (including the role of water), predicting free energy of docking for various ligand-receptor complexes and reconstructing complete free energy surface [126] as well as allosteric effects [127]. This can be visualised using software such as Visual molecular Dynamics (VMD) (Figure 1), allowing the researcher visually interpret results and study the role of the different molecules in the assosciation path. Recently, dynamic docking has been successfully used to reveal cryptic binding sites [128], whose detection is problematic, due to the fact they are not detectable based on protein structure alone, because they might be revealed during the binding process. Despite the outstanding progress of the docking method, there are still methodological barriers to overcome, for example docking to flat receptor surfaces or to intrinsically disordered proteins.
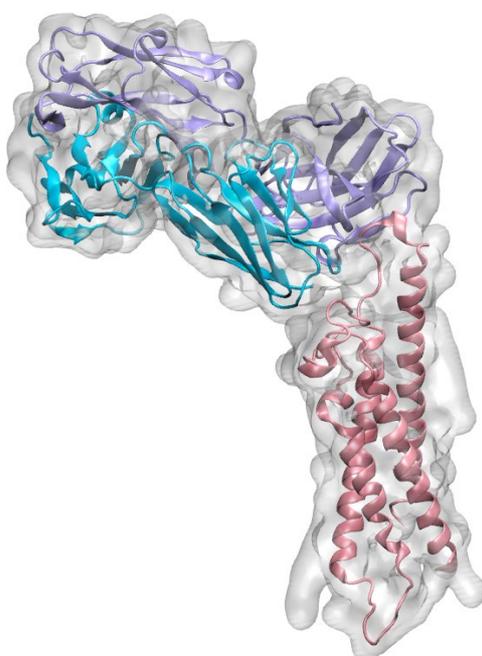
Figure 1: Image showing the binding of the heavy and light Fab chains (purple and blue respectively) of Rituximab bound to CD20 (pink). Showing the binding site of CD20. PDB file 6VJA.pdb [129].

Although sometimes computationally expensive, in general theoretical methods are much faster and much cheaper than the respective experimental approach. Moreover, constant technical development of computers, as well as effort invested to optimise the algorithms make them even more affordable, in terms of time required for conducting appropriate simulation, and the total cost of the research project. On the other hand, theoretical methods offer only plausible predictions of the detailed system behaviour; the hypothesis made based on simulations need to be tested and verified experimentally. Therefore, a combined, theoretical and experimental approach seems to be the most reasonable way to study complex biological and pharmacological relevant systems. The theoretical methods applied at the initial stage of the research have huge potential to drastically narrow the number of hypotheses, that in turn reduces costs and time required for necessary experimental tests.

Moreover, the results from simulations might be successfully used to better understand experimental data [130], which are usually difficult to interpret due to lack of the full control of the system studied. In particular, theoretical methods and predictions have been successfully used to elucidate conformational changes of antibody upon ligand binding [30], as well as details of its dynamic behaviour [131].

Therefore, theoretical methods, with their power to predict detailed behaviour of biological systems, should be an integral part of modern studies requiring a molecular level of understanding of biologically important systems.

4: Drug delivery solutions

Antibody therapy:

In addition to cytotoxic molecules, antibodies can be conjugated to nanoparticles (NPs), and taking advantage of favourable physicochemical properties of NPs [132], these can be used for therapeutic, as well as diagnostic purposes [133, 134]. NPs offer a range of benefits including: enhanced bioavailability/biocompatibility, increased plasma half-life, controlled release at the site where intervention is required, reduced systemic toxicity, and stealth features with appropriate design [135]. A range of different materials can be used, depending on the mechanism of action required, including the use of lipids (micelles, liposomes, non-ionic surfactant vesicles), polymers (dendrimers, polymeric micelles), inorganic matter (silica, metals), drug conjugates (that can include antibodies and aptamers) and viral particles [136]. Different NPs have been adapted for these purposes, with examples related to MM summarised in Table 4.

Table 4: List of antibody-NP conjugates against MM

Target antigen	Antibody-NP	Developer	NP	Reference
CD38	Nanoparticles loaded with S3I-1757 (a STAT3 inhibitor), and then decorated with anti-CD38	University of Alberta	Poly-(ethylene oxide)-block-poly-(-benzyl carboxylate "-caprolactone) (PEO-b-PBCL)	[133]
ABCG2	Epirubicin-loaded lipid microbubbles conjugated with anti-ABCG2 monoclonal antibody	Southeast University	Lipid microbubbles	[137]
ABCG2	ABCG2 monoclonal antibody combined with paclitaxel conjugated with Fe3O4 nanoparticles (NPs)	Southeast University	Oleic acid-coated iron oxide NPs (Fe3O4)	[138]

Aptamer Therapy:

The use of aptamer-NP conjugates in drug delivery benefits from both targeting specificity of aptamers and the unique properties of NPs, including improved pharmacokinetic profile and reduced systemic toxicity of entrapped therapeutic agent. Thus, targeted delivery is optimised through enhanced binding affinity between the aptamer-NP conjugates and their respective target, and subsequent cellular uptake, as well as greater resistance of the complex to degradation [139]. This can also have greater benefits than using drugs directly conjugated to the aptamer, as it could compromise aptamer specificity. For example, Park *et al*. (2015) [140], demonstrated improved specificity and targeting of aptamer-liposome conjugates, entrapping doxorubicin, in comparison to doxorubicin intercalated into the aptamer, due to

possible alterations in aptamer structure. The use of aptamer-NP conjugates in cancer treatment is still in preclinical stages, with studies yet to be reported for MM. However, promising results have been demonstrated for other cancers using different types of NPs, including liposomes [99, 141-143], polymeric NPs [144-147], inorganic NPs [14, 148-150], dendrimers; [151], micelles [152] virus-like particles [153] and nanogels [154].

5: Technology Comparison

Targeted anti-cancer treatments are restricted by the current limitations in available biomarkers, as well as the identification of patients that would benefit from immunotherapy [155]. In order to maximise the efficacy of antibody-based therapies, and identify patients that would most benefit from them, prognostic and predictive biomarkers are required. This makes patient stratification possible, ensuring patients can be placed on appropriate and beneficial treatment regimens, and their responses more easily monitored [155]. While this is still an important challenge to overcome in many cancer types, promising results have been seen in melanoma and non-small cell lung carcinoma (NSCLC) [156]. The overexpression of PD-L1 in these two tumour types has been associated with a poor prognosis, however, when treating these patients with anti-PD-1 antibodies, such as nivolumab or pembrolizumab, progression-free survival, overall survival, response rate and duration of response increased when compared to chemotherapy [156]. This shows that even if the identification of predictive and prognostic biomarkers is a challenge, their identification is crucial to maximise patient benefit.

Aptamers hold great potential for the treatment of MM. The smaller size of aptamers (molecular weight 10-50 kDa) in contrast to mAbs (molecular weight 140-700 kDa), gives aptamers the advantage of tissue penetration [116]. As aptamers are chemically synthesised, microbial contamination during synthesis is avoided, synthesis time is reduced and various chemical modifications can be easily introduced during synthesis [157]. Aptamers can be synthesised to target a variety of immunogenic and non-immunogenic targets [158]. They are stable in variable conditions, and thermal denaturation is reversible where they can return to their original conformation without compromising activity [159]. Antidote aptamers can be developed against therapeutic aptamers to reverse or control their action [27]. The scale up of aptamer production to large scale manufacturing is simple and less expensive with minimum batch to batch variation [160].

However, despite the great benefits that aptamer therapy could provide, there are some challenges. For instance, aptamers have to be chemically modified to avoid digestion by serum nucleases, especially RNA aptamers. It has been argued that RNA aptamers are more susceptible to nuclease degradation than DNA aptamers, despite the preference of RNA aptamers due to their greater flexibility folding into more diverse 3D structures [158, 161]. DNA aptamers are considered resistant to 2'-endonulceases as they lack the 2'-OH groups and have been reported to offer greater stability than their RNA counterparts [162]. In fact, AS1411, which has reached phase II clinical trials, is an unmodified DNA aptamer with proven stability [117, 163]. Although the small size of aptamers allows tissue penetration thereby enhancing targeted delivery, the downside of this property is rapid renal clearance and short half-life. Many aptamers have been modified through pegylation to avoid renal clearance including NOX-A12 and the approved aptamer Macugen® [157]. However, concerns have risen recently regarding the safety and efficacy of using pegylated therapeutics, due to their association with the generation of anti-PEG antibodies [164-166]. Anti-PEG antibodies, which have been found in humans and animals, have been reported to bind to pegylated aptamers, resulting in allergic reactions and inhibited therapeutic activity [167, 168]. In fact, not only are anti-PEG antibodies induced after administration of pegylated aptamers, but they can also pre-exist prior to

treatment causing severe immediate allergic reactions [169, 170]. In addition to the challenges related to identifying aptamer sequences that bind to specific tumour biomarkers, tumour heterogeneity can make aptamer therapy more challenging where specificity and affinity of the developed aptamer to certain tumour biomarkers is compromised [121]. Although the pharmacokinetic, pharmacodynamics and safety profile of aptamers have been reviewed by Kovacevic *et al.* (2018) [167], further clinical research is required as only one aptamer, administered by intravitreal injection in small doses, is currently available for clinical use, with those administered systemically in phase II clinical trials.

Antibodies have been used for decades clinically, and have had an important role in cancer treatment (as seen in section 2), however, they face some limitations. In solid tumours, including extramedullary involvement of MM, poor penetration and heterogeneous distribution of mAbs can lead to reduced therapeutic effectiveness [171]. An improvement in tumour penetration has been achieved through the use of scFvs and chemically linked Fabs, which are much smaller in size and can therefore diffuse faster and easier [172]. Despite their advantage over standard antibodies in terms of tumour penetration, antibody fragments do not solve the issue of heterogeneous distribution, as clearance rates of these smaller molecules is high, with half lives of hours or even minutes [172]. This prevents proper tumour targeting and homogeneous distribution, and therefore supposes a significant challenge to overcome. An alternative approach was adopted by using Chimeric antigen receptor T cells (CART) to target BCMA with humanised single-domain antibody, which has demonstrated great efficacy in Phase 1 trial [173]. As explained earlier, aptamer therapy progress is also hindered by fast clearance rates, as they are rapidly removed due to their small size, highlighting the need of further improvements in technology to fully harness their potential. A second major limitation for antibody therapy is treatment resistance, with ultimately all patients developing resistance to treatment regardless of previous effectiveness [174]. It is impossible to compare antibodies and aptamers in terms of therapy resistance due to the lack of aptamers used in MM therapy as well as trial data. Therefore, while antibody therapy needs to improve in terms of overcoming therapy resistance, it is unclear if aptamer therapy will face the same limitation. This highlights the importance of molecular docking, as it can help understand therapy resistance and aid in the discovery of novel therapeutical targets.

Finally, the production of monoclonal antibodies, via the use of animals is becoming more restricted by the EURL ECVAM's Scientific Advisory Committee (ESAC), with the committee urging for the prioritisation of non-animal derived antibodies and aptamers [175].

6: Clinical Perspective

The median overall survival in MM has improved significantly in recent years. However, prognosis remains variable, with relapse inevitable in almost all patients, particularly in those defined as clinically high-risk. In almost a fifth of patients, MM can still lead to death within the first 3 years from diagnosis [176]. Furthermore, despite the overall toxicity profile of these approaches being considered favourable, a patient will often require multiple treatment strategies in their disease course, and toxicity profiles can be compounded, limiting further therapy. A careful balance, therefore, exists between treatment efficacy and toxicity, which should be individualised for each patient.

Antibody and aptamer therapy are attractive options in MM due to the likely durable responses associated with this drug design and delivery, as described above. However, long-term toxicity, treatment resistance and effects on varying antigen expression through disease progression, will need to be carefully evaluated through clinical trials. In the UK, we are fortunate that clinical trials are developed and delivered on a national platform, through the National Cancer Research Institute (NCRI) and the National Institute of Health Research

(NIHR), where fundamental developments in myeloma care have been generated [177]. However, there are several limitations to an accelerated trial design allowing rapid assessment of pre-clinical drugs into a clinical setting, including funding and delays in trial set-up [178]. It is estimated that time delays from pre-clinical to completion of clinical trial evaluation can be in the region of 10-15 years. In order to allow for novel drug delivery systems as described, these limitations need to be addressed at a national level so that patient outcomes continue to improve in MM.

7: Future perspectives

Monoclonal antibodies have given rise to other, more advanced derivatives, such as chemically linked Fabs, bivalent and trivalent scFvs, nanobodies and fusion proteins [179]. These monoclonal antibody derivatives aim to overcome their predecessor's limitations by enhancing tumour penetration, showing high efficacy rates, enhancing biological response *in vivo* and side effects being highly controllable due to their short half-life [180, 181]. These advances give antibody therapy an exciting and promising future, but further investigation and therapy engineering is still necessary to unfurl their full potential. While successfully commercialised in a few cases, their use in medicine is still limited. Examples of commercialised BiTEs, a type of chemically linked Fabs, include blinatumomab, which is US and EU approved for Philadelphia chromosome-negative relapsed or refractory acute lymphoblastic leukaemia [182], as well as solitomab, which is in clinical trials for colon, gastric, prostate, ovarian, lung, and pancreatic cancer use (NCT00635596) [183].

Despite the advantages of aptamers, there is a lack of commercialised aptamer-based therapies. This could be due to the high popularity of mAbs and economic investments of companies to produce novel antibody-based therapies [184]. However, the key advantages of aptamers cannot be ignored, with a refinement of the technology needed for them to compete with mAbs in medical and scientific fields.

The use of computational modelling methods such as molecular docking for the purpose of drug design has been reviewed in Section 3. In order to illustrate the relevance of these methods, and their potential in drug design and optimisation, the following articles are worth looking at in more detail. In brief, these methods have been used to characterise, model and study antigen-antibody interactions (docking), all of which are explained in detail in [185], where they also describe available databases, methods and future perspectives of the use of bioinformatics towards the discovery of mAb therapies [185]. This article provides a useful guide on the steps required for the characterisation of novel antibodies though simulations, which is a rapidly evolving field in therapy discovery.

Computational methods have also been utilised in the development of aptamers. The combination of structure prediction tools with docking software and MD simulations, results in reliable aptamer/ligand systems that not only correspond to the experimental systems, but can also be optimised in terms of aptamer binding affinity, cross-reactivity and structure modification for increased *in vivo* stability. Examples of aptamers created using these methods for cancer research include A5U and G15U [186], TIM3-Apt1and TIM3-Apt2 [187] and ERaptR1-R10 [188].

Computational modelling methods can be used to study other types of therapies aside from antibodies and aptamers, such as drug-carrier nanopariclue interactions [189] and drug formulation, stability and solubility [190]. This shows the broad applicability of MD, docking and other modelling methods, which will expand and improve as technological advances continue to occur.

8: Executive Summary

- MM is the second most common haematological malignancy in adults, with prognosis being variable and relapse inevitable in almost all patients, despite a number of different therapeutic strategies being available.

- Antibody and aptamer drug loading based therapies offer an alternative to the current proteasome inhibitor, immunomodulatory agent and anti-CD38 antibody approaches.

- ADCs have been developed to specifically target MM cells and deliver cytotoxic agents chemically conjugated to the antibody to kill the cells and to reduce non-cancer tissue damage. While multi-specific antibodies such as BiTEs have also been exploited.

- Similarly, nucleic acid based molecules such as aptamers can also be conjugated to drugs to form ApDCs and ApOCs.

- Instead of use of single drug molecules, nanoparticles loaded with cytotoxic agents can also be designed in conjunction with antibodies and aptamers to effect additional therapeutic delivery options.

- Nanoparticles offer therapeutic and diagnostic avenues of approach and have been used effectively in non-MM cancer treaments.

- The combination of nanoparticles, antibodies and aptamers are attractive options in MM due to the likely durable responses associated with this drug design and delivery method.

- Computational methods such as docking are becoming more prominent and have become reliable and effective sources of novel antibody / aptamer characteristics and structures.

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