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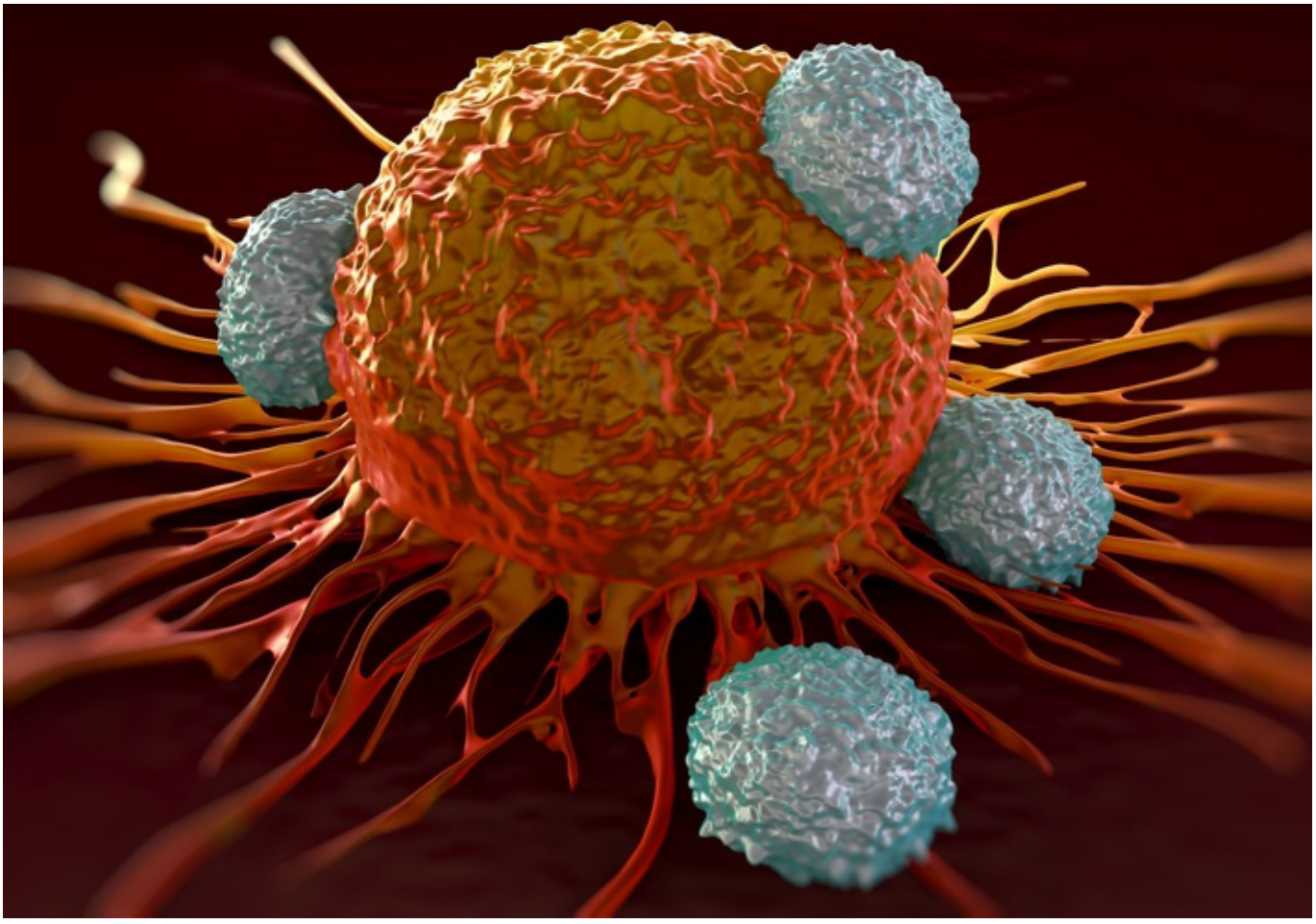

T-cell therapies: Part 2

What comes after CD19 CAR?

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T-cell therapies

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This report forms Part 2 of a detailed review of T-cell therapies. It gives a more detailed and scientific overview of the area to enhance the summary analysis of investment criteria in Part 1. This report has a general technology introduction and four detailed therapy-focused sections.

1: Haematology success: CAR-T and other therapies

This section reviews standard chimeric antigen receptor (CAR) T-cell therapies (CAR-T) for haematological cancers. Leukaemia and subtypes of non-Hodgkin lymphoma (NHL) are well targeted by CD19 (cluster of differentiation 19). Kymriah (Novartis) is approved and Kite's Axi-cel is expected before late November. Other blood cancers need different antigens and present a large and open opportunity.

2: CAR-Ts in solid cancers: Will they work?

The standard CAR T-cell approach needs a clearly defined cancer antigen to work without targeting normal tissue and causing toxicities. These antigens are scarce so in western development, CAR T-cell therapies for solid cancers are rare with leading CD19 companies preferring TCR approaches. There is a lot of solid CAR-T development in China so effective CAR approaches might emerge from there.

3: NKR: Ubiquitous ligands with broad potential

NKR-CAR T-cells target eight ubiquitous stress ligands widely found on cancer cells. The therapy is patented by Celyad. The lead product, CYAD-01, is in a trial (THINK) which Celyad plans to expand to cover five solid and two haematological cancers; multiple myeloma (and AML) may have faster development potential. This approach could be combined with chemotherapy and has allogeneic potential.

4: TCR: Modifying natural for enhanced efficacy

T-cell receptor therapies use engineered T-cell receptors so are not chimeric. Their unique advantage is that they detect specific cancer antigens found only within cells. This means they target cancer, not healthy, tissue. However, their selectivity requires multiple products per cancer and there is a still a risk of side effects.

5: Non-cellular – BITEs and checkpoint inhibitors

Bispecific T-cell engagers are monoclonals so a better fit to pharma business models. They may work in haematological cancers but efficacy in solid tumours needs to be established. Checkpoint inhibitors are a competitive market already but could find much wider extended use in combination with T-cell therapies.

Summary: The proof is in the data

CAR-T therapy works brilliantly in CD19 haematological cancers but other haematological and solid cancers need new approaches to treat 95% of the unmet medical need. It is highly uncertain if CAR-Ts will work in solid cancer. Celyad's NKR technology offers a ubiquitous approach but needs efficacy data. TCRs can work but target restricted patient groups. The other haematological and solid cancer markets offer a massive T-cell opportunity but also many challenges.

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T-cell technologies: Overview of key issues

Tumours, both solid and blood cancers, are presumed to evolve and interact with the immune system in a three-stage process.

- **Eliminate:** New cancer cells are recognised by the immune system as mutated or with genetic damage and killed by $CD8^+$ T-cells or possibly by natural killer (NK) cells. If successful, the 'patient' never knows they had a cancer. This probably happens all the time in most people.
- **Equilibrium:** With selective pressure from the immune system, the only cancer cells that survive have mutated and altered to escape immune detection and destruction. The evolving cancer is now tolerated by the immune system but any aggressive mutants are still detected and destroyed. The tumour is probably still localised or regional.
- **Escape:** Malignant cells have evolved and supress any immune response. The cancer becomes more embryonic (so less controlled in its growth) and starts to metastasise. It makes proteins only normally found in developing embryos – so further marking it out from healthy tissues. It also becomes more heterogeneous with different tumour clones varying their gene expression patterns and displaying different antigens. Immune system cells patrol the tumour mass, but do not attack even though they may recognise tumour cells. Even if one clone type is destroyed by immune action, chemotherapy or radiation, others evolve resistance and escape mechanisms and take their place.

The power and appeal of T-cell therapy approach is that by introducing a precisely targeted attack on the cancer cells, tumours could be moved back down this evolutionary path from escape, to either equilibrium or eliminate. Eliminate might be possible in haematological cancers, but probably will be a less frequent event in solid cancer therapy unless effective combinations are developed.

Elimination of the cancer, true cure, cannot be proven without long-term follow up. T-cell therapies are too new and trialled in too few patients, to be sure of long-term response rates; even in approved CD19 ALL, the long terms remission is yet to be established. In haematological cancers, persistence of the modified T-cells is perceived as crucial for long-term "cure". Persistence might be a long-term toxicity risk factor in solid cancers. The patient's immune system needs to be stimulated to respond to a range of cancer antigens to prevent tumour escape implying combination therapy, for example, with checkpoint inhibitors and standard therapies.

To achieve equilibrium or elimination with minimal toxicity, therapy needs to be on target and on tumour, Exhibit 1. The extent to which this occurs with different therapy options is examined in the report as a key theme across all technologies. These effects are separate from the generally manageable short-term side effects of cytokine release and neurotoxicity associated with standard CAR-T and TCR dosing; NKR dosing is different and has not shown short-term side effects to date.

Exhibit 1: On and off target effects

	On target	Off target
On tumour	This is the aim of the therapy: a specific antigen on a tumour is effectively targeted, delivering cancer cell killing and potential cure. CAR constructs can be engineered to be very strongly binding giving powerful responses. NK receptors already have strong ligand binding.	An aim of all modified T-cell therapies is to get antigen spreading to give lasting immunity. Preconditioning by damaging and degrading the existing immune system may make this harder to achieve. Checkpoint inhibitors rely on this effect as they are not targeted.
Off tumour	The tumour antigen is also present at lower levels on healthy tissue, meaning that the therapy kills healthy cells. This can lead to attacks on other organs that can be difficult to control, cause damage or be fatal.	This is a potential, sometimes fatal, side effect. It is mostly a risk in TCR development, but is a concern for any approach. It means that initial clinical studies are cautious and dose ranging.

Source: Edison Investment Research

Developers seek "**on target, on tumour**" specificity so the CAR therapy directly attacks the tumour cells using the designated antigen target. They also want "**off target, on tumour**" effects, whereby the tumour is targeted by the host immune system using a range of antigen targets. NKR CAR T-cell therapy is designed to try to generate such a response, as seen in preclinical work.

The risks come from off tumour targeting. A T-cell therapy is alive – the transformed T-cells grow, and die – so the cell dose does not necessarily relate to efficacy and CAR T-cells could persist for years. The particular risk limiting standard CAR and BiTEs (also as known checkpoint toxicities) is "**on target, off tumour**" toxicity. This is a side effect of CD19 therapy where both malignant and healthy B-cells are targeted. It is a theoretical risk in NKR therapy in case of infection, inflammation or chemotherapy that might "stress" healthy cells.

TCRs are highly specific therapies but the biology of TCRs means that "**off target, off tumour**" effects are a risk. This is where the modified T-cells unexpectedly and strongly react against a different target on healthy tissue. This should be a rare event but it can occur and has been fatal.

There are three clinical, and one emerging preclinical T-cell approaches discussed in this report.

- **CAR-T:** The original CAR T-cell approach using autologous (from the same person) T-cells where the T-cell receptor is radically modified and becomes chimeric by replacing the external binding domain with antigen-specific antibody binding fragments. Therapies are enhanced by adding extra internal co-stimulatory signalling and by administering aggressive prior chemotherapy targeting the endogenous immune system (preconditioning) and the immune cancer before the new CAR T-cells are infused. Side effects after administration (cytokine release syndrome and neurotoxicity) can often be severe but are mostly manageable.
  CAR-T therapies using CD19 targeting have so far given high response rates in relapsed and refractory leukaemias and lymphomas enabling them to be used as a bridge to stem cell transplantation or possible long-term cure. However, relapse rates might be significant and so far, only short-term results are published.
  The outcome in multiple myeloma, and other haematological cancers using non-CD19 antigens, is less apparent so far. There is a clear CAR-T antigen target, BCMA, in multiple myeloma and promising early results. Multiple myeloma (MM) therapy has a variety of therapy options including CD123 and NKR ligands although BCMA is in the lead. Data on T-cell therapies for acute myeloid leukaemia is still lacking.
  However, very different cancer antigens will need to be found to move CAR-T into solid cancers. Each solid cancer target is often a new development project. Many "cancer" antigens are also found on normal tissues, although at much lower levels, so can cause side effects. It is possible that CAR-T CD19 for leukaemias and potentially BCMA for MM might prove to be special cases. For the majority of cancers, CAR-T might be sub-optimal or not work at all. The dual CAR-T approach being developed by Autolus may prevent cancer cells escaping by the loss of the targeted antigen and might give improve cancer targeting.
- **NKR CAR:** A proprietary approach from Celyad that inserts a chimeric, augmented natural killer group 2D (NKG2D) receptor into T-cells. Tissues that are infected or inflamed, including genetically damaged cancer cells, display a range of NKG2D ligands as part of a normal "stress" response. As NKG2D ligands are rarely expressed on normal cells, this enables NKR CAR T-cells to discriminate between cancerous and normal cells. As the ligands are ubiquitous, the same NKG2D CAR construct should target multiple tumour types (THINK study). However, efficacy needs to be established and will vary across indications. Any side effects at higher doses are not yet known - although none have been seen to date. The approach does not currently use preconditioning, so has both low side effects and low, long-term toxicity risk. In a realistic solid cancer trial (SHRINK), NKR CART therapy is being tested after chemotherapy.
- **TCR:** These T-cell therapies use optimised T-cell receptors (TCRs) that are inserted into harvested T-cells. The method is highly tumour specific and detects cancer antigen fragments from proteins that are only within cells; these are invisible to CAR T-cells as CAR-T (and NKR T-cells) only bind surface ligands. However, specific TCR T-cells are limited to subsets of the potential patients. This restricts the market for each TCR product and means that multiple products need to be developed to cover most patients in any indication. However, one TCR

type might apply to several cancer types. There is also a risk of unpredictable and serious potential side effects. TCR T-cells are used, so far, with preconditioning.

- **CAR NK-cells:** This is a concept which puts an antibody-type, standard CAR into a natural killer cell. Whether this CAR NK approach will work is unclear. As early-stage concepts, they are not considered further in this report; Bellicum has a small early trial running.

There are two non-cellular approaches that utilise endogenous T-cells but do not require T-cell harvesting and modification. Both use monoclonal antibody technologies and production systems. This makes them very attractive to major pharmaceutical companies as they are much cheaper to develop and can be produced at scale as off-the-shelf, mass market therapies.

- **BiTEs (bispecific T-cell engagers):** These are antibody-like therapies to link any endogenous T-cell to a cancer cell and trigger an attack. In therapy, this could give a "CAR-like" response without manipulating T-cells. However, BiTEs are large proteins that will have difficulty passively penetrating solid tumours. This means they are likely to be of most use in blood cancers where the cancer cells are accessible and there are plenty of passing T-cells to grab and activate. Potentially, this makes them lower-cost competitors to haematological CAR T-cell therapies and a low threat to solid cancer CAR T-cell developments.
- **Checkpoint inhibitors:** These are marketed products rightly seen as breakthrough immune therapies. If they break immune tolerance against a patient's tumour, then long-term remission or cure can be obtained. However, tolerance is not fully broken in most patients and the therapies as standalone options only work in the most immunogenic cancers like lung, skin and bladder. They could be combination therapies with solid CAR T-cell approaches as they may facilitate antigen spreading, so generating a more powerful endogenous immune response. This needs to be established but is a likely development route for many T-cell approaches.

Background on these technologies is given in Exhibit 2 and more detail and diagrams are in each section where required.

Manufacturing – are we nearly there yet?

An aspect not fully considered in detail is the supply and pricing of these therapies. Manufacturing is currently, and at best, a standardised process not a standardised product. There are some general issues behind this.

- **Provision of adequate virus:** Current viral production systems are low yielding and very manual. CAR and TCR companies buy virus from third-party manufacturers.
- **The reliability of manufacturing:** Cells for autologous products are harvested from patients who are very ill. Hence the quality of the starting cell is variable and manufacturing is not fully predictable though getting better. Regulators like standardised products and doses.
- **Cost**: Low volumes, high facility overhead costs and a process that is labour intensive mean T-cell therapies are expensive. Virus is very costly currently.

One solution is allogeneic therapy where standardised cell lines are prepared and transformed in bulk. This would make therapy available immediately or within a few days and probably much cheaper. Celyad has a strong core patent on producing allogeneic cells; Novartis bought a non-exclusive licence in May 2017 for US$96m plus royalties. Cellectis is in clinical studies with UCART123 although the trial encountered side effects is on hold. Allogeneic production will be reviewed more fully as it is an emerging area that is likely to become more important as it is potentially critical to expanding the use of T-cell therapies especially into solid cancers.

Exhibit 2: Technical summary of technologies

Name	Technical description	Commentary
Standard CAR-T technology	Standard CAR is still very new and evolving in the haematological cancer space with the first approved product. It uses an antibody like binding region that sits on the surface of the T-cell. This binds an antigen on the surface of cancer cells. Once bound, the T-cell internal signalling systems are activated to drive the cell killing response. To do this, the antibody-like external part is coupled with an internal "CD3ζ" signalling domain plus various other co-stimulatory molecules.	There are conflicting patent claims on these technologies. The whole genetic package for this construct needs to be implanted in T-cells harvested from the patient. To do this, various viral systems are used. Once infected, the cells are cultured to get more of them and then reinfused into the patient. This also creates logistical and quality control challenges. It is a complex process that takes some weeks.
NKG2D natural killer receptor-based CAR T-cell technology (NKR-CAR)	This takes one receptor, the natural killer receptor-based CAR T-cell group 2D receptor (NKG2D), augments it with a CD3ζ internal signalling domain to boost its activation signal (so it is CAR-like) and complexes it with its normal co-stimulatory molecule (DAP10) found on all T-cells. This gives a second-generation effect CAR but avoids the complexities and patents of added CD28 or 4-1BB co-stimulatory domains. The NKR CAR is inserted into isolated patient T-cells using a retroviral viral vector followed by culture and reinfusion. There are eight known NKG2D ligands (MICA, MICB and ULBP numbers 1-6; also called antigens), of which four have been studied in detail.	The current uncertainties for this approach are the need to establish clear signs of efficacy in one or more cancer types, both haematological and solid. There is preclinical evidence to suggest that toxic dose levels are very much higher than those planned in clinical development. The cells show low persistence; precondition is not used so the cells do not expand much after infusion. In preclinical models, the host (patient) immune system seems to become engaged to maintain long-term cancer control. NKG2D in its original form is used by natural killer cells (another killer immune cell type) to detect cells infected by viruses so they can be killed as part of the rapid response, innate immune system.
T-cell receptors (TCR)	TCRs are complicated, multiprotein natural receptors used by killer T-cells to find infected and non-self-cells. TCRs have a significant advantage as they bind small fragments of internal proteins (peptides) displayed on MHC molecules on the cell surface. A TCR can detect a single mutation in one internal cell protein and unleash the T-cell destructive power as a result. For cancer therapy, a TCR against a specific antigen is selected, its affinity optimised and the novel TCR genes inserted into harvested patient T-cells. These are cultured and reinfused. Optimising a TCR is a delicate business. Too low an affinity and efficacy is reduced with more risk of toxicity. Too high and the T-cell carrying it sticks to the cells and deactivates; T-cells like to roam around. This is unlike CAR and BiTE constructs where the aim is to get tight binding. It is unlike NKR CAR where the NKG2D receptor is optimised though evolution.	TCRs seem ideal – a new set of specific cancer antigens. As they never appear on the cell surface as intact proteins, internal cancer antigens cannot be detected by CAR T-cell and BiTEs. However, every TCR is HLA specific and there is enormous diversity in the HLA (tissue) types in the human population. Hence, one TCR is limited to patients with that HLA type. At best, this is 50% of the population if HLA- A2 is chosen, as it normally is. This means that multiple TCR varieties are needed to cover most of any cancer indication which is probably economically unviable under current development paradigms and regulations. TCRs can also recognise other targets on normal cells although this is not predictable and can be missed in screening. This has led to serious and sometimes fatal side effects. There are few TCR companies as a result of the development complexities. The first major TCR data set is due in 2017.
CAR NK-cells	A CAR-type construct using CAR T-cell technology but inserted into harvested natural killer cells not T-cells. The NK-CAR cells are cultured and re-infused. Currently preclinical. Will take some years to gain any clinical efficacy data. Note: this is the reverse of the NKR T-cell approach.	NK cells are found in very low numbers in solid tumours. They are hard to culture while retaining activity so getting adequate human doses may be difficult. NK-cells, as innate immune cells, are tightly regulated to stop attacks on "self" cells as their activity is downregulated by "self" HLA molecules on target cells. Cancer cells usually retain some HLAs to prevent NK attack.
Bispecific T-cell engagers	BiTEs are engineered, large antibody like proteins that are infused into the blood and passively circulate. They have two binding arms designed to bind separate targets so are bi-specific. One arm of the BiTE anchors to a cancer cell; the other grabs a passing T-cell to activate the killing response. There are an enormous number of possible designs but all those considered in this report have one CD3 binding arm to bind and activate a killer T-cell. The other arm can be an antibody-like molecule binding a cancer antigen, a TCR (see below) against internal cancer antigens or even an NK cell receptor like B7-H6.	Like anti-cancer monoclonal antibodies, they are maybe of most use in haematological cancers; preclinical evidence suggests utility in some solid tumour types. However, they passively rely on itinerant T-cells for efficacy and their ability to access cancer cells embedded in solid tumour masses is uncertain. The choice of antigen bound by the other arm also determines efficacy and side effects and here the paucity of antigen choice has limited commercial development. As efficacy is likely to be less spectacular than CAR T-cells, larger and longer trials seem to be needed. Large pharma seems to be investing in these as they fit into their development and commercial structures.
Checkpoint inhibitors	These are a range of approved therapeutic antibodies designed to overcome immune tolerance to cancer. A CTLA-4 inhibitor (only one approved) allows a diverse immune T-cell response to develop and works well in melanoma or checkpoint inhibitors. Other approved checkpoint inhibitors bind either the PD1 receptor or PD-L1 ligand. Blocking PD1/PD-L1 stops the deactivation of T-cells. Various PD1/PD-L1 antibodies are approved for various immunogenic cancers.	Main markets include non-small cell lung cancer, bladder cancer and melanoma. With marketed products, this is a fiercely contested market.

Source: Edison Investment Research

Success factors in T-cell therapy

The first wave of CD19 CAR-T cell therapies have displayed remarkable efficacy in specific haematological cancers, notably acute lymphoblastic leukaemia (ALL) and diffuse large B-cell lymphoma DLBCL (the common form, about 25% of cases, of non-Hodgkin lymphoma).

The range of factors at play makes it difficult to pin down the key technologies and issues that clinicians and investors should focus on. Dr Stanley Frankel, vice president and the head of immune-oncology clinical R&D at Celgene has highlighted a range of variables that are key in determining toxicity and efficacy.

- **Product issues around therapy design**
  - Starting cell viability and quality
  - Technique utilised for gene insertion (commonly a vector like lentiviral virus)
  - Choice of construct components including singly chain variable fragment (scFv), spacer, transmembrane domain and co-stimulatory domain
  - Choice of target (CD19 etc)
- **Protocol for administering cells**
  - Cell dose
  - Dose split/number
  - Lymphodepletion (Preconditioning, a key source of direct, sometimes fatal, toxicities.)
- **Patient factors in response**
  - Disease burden
  - Target accessibility
  - Age
  - Biological variables including other mutations (eg PD-1/PDL-1)

These therapies have been developed at an impressive speed, but many questions remain unanswered, including control of severe side effects, durability of responses, and cost.

Potential efficacy

As yet, the efficacy of T-cell therapies in solid tumours is not known but it is unlikely that a solid cancer will respond in the same way or with the same spectacular response rates, as in haematological CD19 cancers or as with aggressive chemotherapy. The Novartis ELIANA study for Kymriah approval in acute lymphocytic leukemia **(**ALL) found that 83% of patients responded.

A review by Newick et al (2016), based at the University of Pennsylvania, a leading centre for CAR therapy development, clarifies the multifactorial difficulties involved in solid tumour therapy beyond the major issue of antigen selectivity, Exhibit 3. As yet, with limited clinical data, it is not feasible to assess how significant many of these may be – or what T-cell developers can do about these.

Given the major need in solid cancers, lower response rates but with a high proportion of patients achieving stable disease would offer significant clinical gains. For example, pancreatic and ovarian cancers are mostly found late and are very intractable so even stable disease or partial response with prolonged survival would be a major advance. Clinicians now assess solid cancer therapies by immediate responses (response evaluation criteria in solid tumours: RECIST). For chemotherapy, this is essential; the active, highly toxic, agents are only in the patient's body for short periods so therapy has to destroy cancer cells quickly. But in cancer, immediate responses do not necessarily translate into major survival gains as resistant residual cancer cells remain.

Exhibit 3: Major issues in solid tumour therapy development

Issue	Technical factors	Comment
The need for infused, modified T-cells to migrate to tumours	First, infused T-cells tend to become stuck for some hours in the capillary bed of the lungs. Hence, any off tumour toxicity in lung tissue could be exacerbated. Once the T-cells escape from the lung they use chemokine receptors to locate tumour sites. Chemokines are chemical signals but the chemokines released by the tumour need to match the receptors carried by the T-cells. If they do, the T-cells migrate in large numbers to the tumour and infiltrate from the blood into the solid tumour mass. The usual CAR T-cell chemokine receptors are CXCR3 and CCR5. Lung cancers tend to produce the ligand CCL2. A T-cell with a CCR2b receptor move better to lung tumours.	There are trials, including the LINK trial proposed by Celyad, which use intra-tumour injection. This is fine for head and neck cancers and cutaneous melanomas but less easy for internal tumours unless done during surgery and impossible for small, multiple secondary tumours. Solid tumours generally have poor blood supply and can be randomly located. In solid tumours, the cells are also embedded in the extracellular matrix so can be more difficult to access. The selection of CAR T-cells that match the tumour chemokine profile (if known) might be possible but adds complexity and cost.
T-cell infiltration of tumours	Tumours have a higher internal pressure than the circulatory system. This is a physical barrier to any T-cell moving into the tumour. Tumours are composed of both cancer and normal cells imbedded in an extracellular matrix, a composite of sugars and proteins. T-cells need to enzymatically dissolve this matrix to move through the tumour and access cells.	T-cells naturally access and infiltrate tumours especially if attracted by cytokine and chemokines. In some preclinical models, CAR T-cells have had additional heparanase enzymes incorporated to dissolve and so "tunnel" through the matrix. These have not been tested in trials.
The need to overcome Treg and TFGβ immune suppression	There are multiple factors operating here. IL2 stimulates CD8+ T-cells at high dose (including CAR T-cells but at low doses preferentially stimulates Tregs (regulatory T-cells) so preventing T-cell activation and expansion. Tregs use various methods, including secretion of Transforming growth factor beta (TFGβ), to supress CD*+ T-cell activity.	Tregs are a relatively small component of the CD4+ cell population but higher levels correlate with a worse prognosis. Tregs may be ablated by preconditioning so enabling an unrestrained CAR-T or TCR T-cell tumour attack. Tregs also express NK ligands so may be eliminated by NKR CAR T-cells - although this has not been established clinically. Using cytokines like IL12 (Juno's "armoured" CARs for example) avoids IL2 SPEAR technology (Adaptimmune) adds soluble TFGβ receptor genes into modified TCR T-cells to block TFGβ signalling by cancer and Treg cells.
Checkpoint ligands may downregulate the response	Cancer cells often express PD-L1 although the diagnostic measurement of these is problematic. T-cells have PD-1 receptors so can be triggered into apoptosis by PD-L1 ligands on cancer cells. This is a common tumour survival strategy.	In CPI development, the correlation between PD-L1 tumour levels and efficacy is not clear with responses seen in very low (to 5% expression) or negative. PD-L1 tumours. CAR and TCR T-cell and PD1/PD-L1 combination therapies are already being explored and this route is more likely in future.
Low amino acid level in tumours	It is known that tumours make high levels of an enzyme, indoleamine 2,3-dioxygenase (IDO) that metabolises the essential amino acid tryptophan to kynurenine. Low tryptophan and high kynurenine levels are said to supress T-cells. An animal model where IDO+ tumours were used was not controlled by T-cells whereas tumour grafts with no IDO was eliminated. Preconditioning may reduce IDO levels Ninomiya et al (2013)	This is a commonly stated theory but direct measurement of T-cells *in vitro* shows that the tryptophan levels need to be well under half of the natural *in vivo* 50nM concentration to have this effect and kynurenine levels need to be tenfold or even higher than measured physiologically. Nonetheless, some effect seems to be occurring perhaps at a very localised level in the tumour mass.
Lack of oxygen (hypoxia)	T-cells need energy to infiltrate tumours and mount attacks on cancer cells. Tumours, due to demand from the cancer cells and poor vasculature, have low oxygen levels which impair T-cell function. Large tumour masses may be hypoxic in the centre leading to high levels of lactic acid and a hostile, acidic environment (blood is normally mildly alkaline).	CAR T-cells and TCR therapies can do little on this. BiTEs will have trouble penetrating poorly vascularised tumours anyway as these are large proteins relying on passive transport. NKR CAR T-cell approaches could benefit as these conditions seem to lead to tumour vascular epithelial cells expressing NKG2D ligands and there is preclinical evidence that stressed normal blood vessels inside tumour mases can be targeted and destroyed by NKR therapy.

Source: Edison Investment Research, cited references including Newick et al (2016)

A more robust T-cell therapy clinical metric, as with any therapy, is overall survival, but such data could take years to produce. Criteria based on disease stability and longer-term responses might be more appropriate for therapies that may persist for long periods in patients and aims to generate generalised immune control. For example, Keytruda, the checkpoint inhibitor (CPI) given with chemotherapy for non-small cell lung cancer (nsclc) gave a 55% partial response vs 29% on chemotherapy alone, seen as a strong result in this indication.

In CAR therapy in solid tumours, Celyad has noted a potential response in an AML patient at a low single dose and, in the THINK study, has seen two stable disease cases in metastatic colorectal cancer at the lowest dose level (three doses given).

Kite Pharma has reported that on a NCI TCR dose escalation study in advanced cancers, three patients of nine (30%) at the target dose showed tumour regression; another patient with cervical cancer had a complete remission at a lower dose.

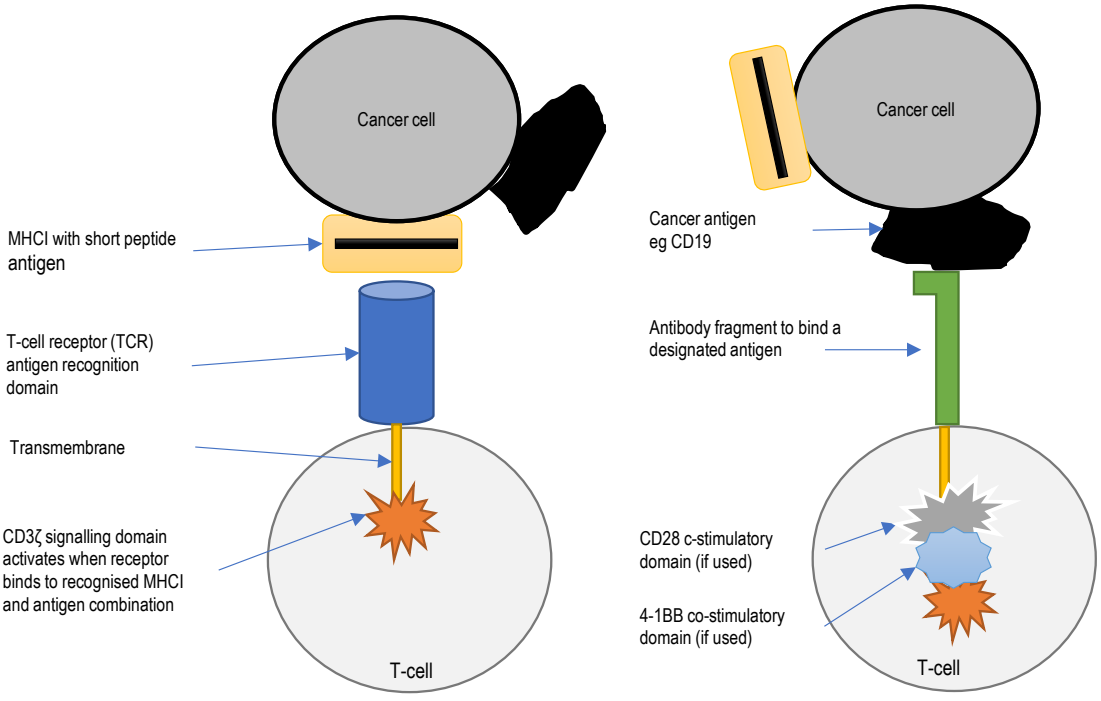
Section 1: Haematological cancers

This section looks at standard chimeric antigen receptor T-cell therapies (CAR-T). The technologies and clinical lessons learnt to date affect the development of other therapy approaches although these will diverge over time. We then turn our focus on CD19 CAR-T in leukaemias and lymphomas. This might be a special case where the antigen is highly specific to the target tissue and the cancer is accessible enough for fast clinical responses. These still remain highly customised and still unpredictable therapies. Multiple myeloma and myeloid cancers present different challenges and are a more open field with diverse competing technologies.

Making a CAR-T

The leading approach to modifying T-cells is to insert a CAR into a CD8+ "killer" T-cell. To date, this approach has produced 83% response rates in clinical trials, albeit in a niche group of patients. A CAR combines a foreign (chimeric) recognition element with the target cell killing ability of a T-cell. The genes for this construct are inserted into the patient's T-cells which then express (make) the CAR and locate it on their cell surfaces. Exhibit 4 shows a highly simplified schematic.

Exhibit 4: Transforming a TCR into a CAR-T



Source: Edison Investment Research. Note that a "natural" TCR will have six CD3 proteins associated. Only one CD3ζ is shown. CD28, OX40 or 4-1BB may or may not be used. Second generation CAR-T uses one; third generation CAR-T uses two. Kymriah (second generation) uses 4-1BB.

The recognition element commonly comprises a fusion of a single-chain variable fragment (scFv) (an antibody fragment which replaces the original TCR recognition domain) with a flexible external spacer and a transmembrane domain that is in turn linked to an internal stimulatory domain. Internal signalling is augmented with one or two extra co-stimulatory domains to get enhanced efficacy and therapeutic cell survival.

Due to the speed at which the field is moving, multiple approaches exist in the design, construction and manufacturing of CAR T-cells. The main components of the construct are the scFv, spacer and

stimulatory domains. It is worth noting that in the July 2017 FDA advisory committee panel for Novartis's Kymriah it was evident how little is conclusively known about the driving factors of efficacy and safety.

Co-stimulation: The key to efficacy and persistence

In first generation CAR cells, CD3ζ was the only stimulatory component. While this proved effective in inducing cell death of a bound target, persistence and generation of a sustained T-cell response was elusive.

In 1998, Krause et al (1998) showed that adding CD28 to scFvs gave better efficacy and persistence. Accordingly, second generation CAR-T therapies use dual stimulation of the T-cell to obtain sustained and effective tumour cell killing. CD28 co-stimulation was the first CD protein to be used and is utilised in Kite Pharma's Axi-Cel (KTE-C19). Since then, 4-1BB co-stimulation originally a type II transmembrane protein has been developed (reviewed by Bartkowiak and Curran (2015)). 4-1BB is used in Kymriah. A BCMA-targeted CAR-T therapy for MM (bb2121, Bluebird) also uses 4-1BB co-stimulation. Third generation CAR-T use two or more co-stimulatory domains. Some of the leading CAR-T products and their co-stimulatory domains are outlined in Exhibit 5.

One theory is that CD28 generates quicker CAR-T cell proliferation than 4-1BB but the effect tends to be short lived, while 4-1BB has less immediate potency in generating cell expansion but better supports longer-term persistence. Note that co-stimulatory domains are an area where many patents have been filed. This might influence CAR-T design as much as technical considerations.

The NKR CAR T-cell approach of Celyad uses a different co-stimulatory route with DNA activating Protein 10 (DAP10) as a natural costimulatory to the NRK receptor. DAP10 is already present in T-cells. This is discussed more in Section 3.

A customised process, not just another drug and target

CAR-T therapy production is far removed from classical drug manufacturing as it involves the modification of either a patient's own cells (autologous) or a donor's (allogeneic). Manufacturing will be key to the success of these therapies. The first wave of CAR-T cells are autologous products where cells are extracted from a patient and re-administered back into them after modification to express the required CAR construct. This process consists of multiple complex steps and to date, companies have kept processes confidential.

One of the key components is how the genes of choice are inserted into the T-cells. Commonly, this is done by utilising viral vectors like lentiviral or retroviral. Variations in approach typically depend on the underlying technology and the relevant freedoms to operate. One concern with vectors is that gene inserts are semi random in nature and as such, they could result in insertional mutagenesis to become oncogenic. Therefore, the manufacture of vectors requires stringent safety protocols. In Novartis's recent advisory committee panel at the FDA these concerns were raised and the long-term follow up of Kymriah patients is planned.

Dosing remains a key consideration, while the leaders in the field appear to have (for now) mostly settled on a common dosing range (~10^6cells/kg), factors such as cell quality, expansion and cancer burden remain key when considering dosing. Dosing, for example in product/Kg as is common with many classical drugs may not be suitable for CAR-T cells; however, it is currently the approach taken. NKR CAR T-cell therapy is using a fixed dose. Key questions about the quality of the starting cells and how both expansion and disease burden appears to affect efficacy and safety still need to be fully answered. Novartis in its recent advisory committee panel at the FDA gave the most public detail yet on what manufacturing defines its CAR-T response. It saw no correlation between CAR viral transduction and potency in vitro to efficacy or safety. While the small patient

numbers tested to date make any correlations more difficult to observe, this data is indicative of how little is still understood about CAR-T mechanisms of action.

This summary highlights the complexities of customised CAR-T production.

- **12 to 2 weeks beforehand**
  - Screening and selection of eligible patients; once selected, patients have a blood sample taken. Disease state and immune components are measured.
  - Leukapheresis of the blood sample (separation of white blood cells, in this case T-cells from the patients' blood by a specialist machine).
  - T-cells are modified by inserting the CAR construct genes. Usually performed with either lentiviral or retroviral vectors so the genes integrate into the genome for long-term stability.
  - CAR T-cells are stimulated to expand (increase in concentration).
  - Quality assessment.
  - CAR-T cells are then stored and prepared for infusion (if cells are to be used fresh, less quality assessment is needed and there is a shorter time to reinfusion).
- **8 days before CAR T-cell infusion**
  - Reassess patients' disease state and immune markers.
- **7 to 1 days before CAR-T cell infusion**
  - Patients undergo lymphodepletion (destruction of a patient's immune system) to enable the CAR T-cells to act uninhibited.
- **CAR-T infusion**
  - Typically, infusion of a single CAR-T dose; however, multiple and staggered dosing is being tested to reduce post infusion side effects.
- **Ongoing post infusion**
  - Assess safety/efficacy (ongoing). Typical efficacy assessment involves monitoring for partial and complete responses, overall survival and minimum residual disease state. Short-term safety, in particular in relation to cytokine release syndrome and neurotoxicity is carefully monitored. Safety arising from insertional mutagenesis or replication competent viral vectors is low but a long-term concern.

A variety of nuances exist in manufacturing between different companies, most of which are not disclosed. For example, bluebird spent significant time working out how it could only get the healthiest T-cells, how it could design the constructs to promote durability and which scFv moiety it should utilise.

Using CAR T-cell therapies

The design, construction and manufacturing of CAR T-cells are of obvious importance. However, understanding a patient's disease burden, any preconditioning needed and the correct dose could prove equally important. Cells are expected to expand by several orders of magnitude or more after administration, so preconditioning is needed. The extent of immediate expansion looks to be a key factor in efficacy but the inter-relationships between dose, expansion, side effects, efficacy and safety are elusive currently.

Preconditioning

Preconditioning is a form of lymphodepletion. Typically, cyclophosphamide and fludarabine have been utilised, in combination or as monotherapies. Cyclophosphamide concentrations of between 100mg/m2 and 2000mg/m2 (Exhibit 5) have been utilised, while fludarabine is frequently employed

at 30mg/m2. However, the exact relationships between efficacy, safety and dose are still elusive. Preconditioning is essential for the efficacy of haematological CAR-T therapies. It is also used in the trial of TCR T-cell therapies but not in NKR CAR trials. Preconditioning has two effects:

- First, by depleting the host immune system, it gives the CAR T-cells immunological space to expand. Expansion of CAR T-cells within patients is key to promoting efficacy; however, rapid expansion is associated with often deadly side effects. Regulatory T-cells (Tregs) normally limit T-cell expansion, but they are also ablated by preconditioning so do not restrict the initial expansion. As they recover, they reassert control.
- Second, immune system cancers like lymphoid leukaemia are susceptible to cyclophosphamide and fludarabine so the preconditioning also depletes these cancers.
  - **Cyclophosphamide**, an oral anti-cancer agent that attacks DNA, is widely used to treat lymphomas, multiple myeloma and leukaemias. It is also used in the treatment of ovarian and breast cancers.
  - **Fludarabine** is an infusion indicated for B-cell refractory chronic lymphocytic leukaemia (CLL). It was used in Juno's now discontinued ROCKET trial of JCAR015 and blamed for initial deaths. However, this does not seem to have been the cause as further deaths occurred after its use ceased

Fludarabine appears in some cases to be central to CAR-T response rates in B-cell cancers like ALL and Diffuse large B cell lymphoma (DLBCL), although it is not indicated for those conditions. Data from a 32-patient trial with r/r B cell non-Hodgkin lymphoma demonstrated that a cyclophosphamide (cy)/fludarabine (flu) regimen demonstrated a complete response (CR) rate of up to 64% compared to cyclophosphamide alone with a CR of 8%. The authors believed the cy/flu combination minimised the immune response to the murine scFv used in the CAR-T therapy. Humanised CAR T-cells or other approaches could minimise the need for toxic chemotherapeutic agents.

Note that outside of haematological cancers, preconditioning will have an immune system ablation effect but will not necessarily attack the cancer. Cyclophosphamide might be effective. If Fludarabine is applied to other cancer types, it is not certain why depleting B-cells will be of any use in encouraging the growth of modified T-cells unless a murine scFv CAR is used.

Control of expansion, whether through dose, analysis of disease burden, correct choice of co-stimulatory domains, or even additional regulatory genes (eg suicide genes) will be key to finding that balance. While the drivers for long-term persistence and prolonged remissions are still not solidly defined, the humanisation of scFv and choice of co-stimulatory domain are likely to play defining roles.

Short-term safety, in particular with relation to cytokine release syndrome (CRS) and neurotoxicity is a concern (see Kroschinsky et al (2017)), although the FDA in considering Kymriah, did not focus on such issues, possibly due to the very high initial response rates. Multiple deaths associated with both have been reported and while management of CRS appears to be viable, neurotoxicity problems in many CAR-T products remain. It is apparent that as clinicians become more aware of the earlier signs of CRS and neurotoxicity, they are learning to treat patients better.

In vivo expansion

Data to date demonstrate that expansion of CAR-T products may be a driving factor in efficacy and safety. In vivo (in the organism, in this case human) T-cells will mobilise upon encountering antigen fragments commonly presented by dendritic cells (antigen presenting cells). T-cell mobilisation involves many factors including the recruitment of macrophages (type of white blood cell that digests foreign substances like cancer cells), neutrophils (type of white blood cell that mediates immune response against infectious microorganisms), other lymphocytes (includes a range of white blood cells like natural killer cells, T-cells and B-cells) and cytokines (secreted proteins that affect

communication between cells stimulating growth, movement and cell death). This activation of the immune system, particularly new T-cells, or CAR-T cells generates a significant immune response. This immune response is uninhibited by regulatory factors and competing T-cells following lymphodepletion can see CAR-T numbers increase by orders of magnitude. As they increase in number, they recruit more immune components driving the immune system into overdrive. In the best case, they completely destroy the cancer.

However, this rapid expansion can also drive what is known as cytokine release syndrome (CRS) where the patient's immune system begins damaging their body through a systemic inflammatory response; deaths have been reported in the most severe cases. This is linked to high levels of Interleukin 6 (IL-6) being generated by the T-cells.

CRS can be quickly reversed by injecting RoActemra (Actemra US) (tocilizumab, Roche). Tocilizumab is a monoclonal antibody that binds and blocks the IL-6 receptor. It was already approved for use in rheumatoid arthritis and in August 2017 was FDA approved to treat CRS.

Control genes and suicide switches

The ability to genetically modify T-cells means that genes to kill or control the response of a CAR T-cell can be inserted. Various systems have been trialled in both preclinical and clinical trials that utilise 'suicide' genes to kill the response of CAR T-cells. Any serious adverse events could be limited by the activation of these genes. However, the onset of severe neurotoxicity and CRS is quick while the activation of these genes takes time and may not be 100% efficient; potentially meaning the activation of the therapy suicide genes may come too late to save the patient.

One of the most promising to date is the utilisation of caspase 9 Stasi et al (2011). Bellicum has a version of this technology called CaspaCIDe. If a small molecule drug, rimiducid, is given the natural apoptosis process is activated, killing the cell. This is being used as a safeguard against graft vs host disease in mismatched stem cell transplant (BPX-501) and in the development of BPX-701, a TCR therapy where off target, off tumour side effects can be lethal. It is also used in an academic trial with CAR-NK cells (NCT03056339). This approach can stop side effects within a claimed 30 minutes. However, despite this remarkable turnaround in toxicity, concerns remained that in other engineered T-cell populations like CAR-T, side effects could remain as a minority population unaffected by the activation of the suicide gene. Cellectis has a suicide gene technology. Academic trials include NCT02274584, NCT02414269, and NCT01822652.

Bellicum has developed GoCART technology to control the CAR-T cell response. In GoCART, the co-stimulatory molecule is separate from the CAR. This costimulatory molecule must dimerise to operate. This only occurs if the small molecule rimiducid (a small, non-drug molecule) is present. Consequently, the CAR T-cells are only active if they bind ligand and if there is an adequate concentration of rimiducid. This should regulate CAR T-cell expansion and control side effects.

Further generations of CARs

Numerous next generation CAR-T approaches are currently being trialled that aim to improve on the current generation. These include inhibitory CAR-T cells that have a second binding domain that will not activate upon the detection of healthy cells; masked CAR-T cells that only activate in the tumour microenvironments; and switchable CAR-T cells that can be turned on and off. However, as the latest clinical data is demonstrating, safety increases may come from better clinical understanding of these technologies..

Patents and orphans: Exclusivity may prove difficult

The patent space is still subject to significant disputes as companies seek to gain an edge. The status of any of these disputes and the applicability and expiry of these and any derivative patents is unknown to Edison. These types of disputes are generally resolved, eventually, with licensing deals as strict enforcement of patents prevents beneficial therapies from being marketed. However, fees and royalty rates can be high and there will be significant legal costs.

One of the key patents from the Sloan Kettering Institute for Cancer Research (US Patent 7,446,190) has been subject to multiple litigations; it is titled "Nucleic acids encoding chimeric T cell receptors" as it covers a range of key CAR-T features including use of CD28 co-stimulatory domains. Juno Therapeutics licensed the patent. In April 2015, Novartis (including its partner, University of Pennsylvania) paid $12.25m to Juno and St. Jude Children's Research Hospital plus future milestones and mid-single-digit royalties on US sales of CAR-T products. Kite Pharma filed an inter-party review to the US Patent office in an attempt to invalidate the patent; however, in December 2016, the patent office ruled that the patent held. With the Gilead acquisition ongoing and a KTE-C19 (Axi-cel) approval anticipated, we think a deal should be expected soon.

Kite holds the "Roberts" patent covering use of scFv in CAR-T US. Patent Number 6,319,494, and exclusively licensed the original CAR patent by Dr Zelig Eshhar U.S. Patent No. 7,741,465.

While these cases highlight the legal battles between companies, one of the key fights may come with regards to market exclusivity and orphan status controlled by regulatory bodies. A workshop at the EMA from the committee for advanced therapies highlighted some of the challenges that companies may face in regards to market exclusivity.

Orphan designation under the US Orphan Drug Act remains critical to ensuring therapies for rare diseases are developed. Orphan designations vary but <200,000 cases is the US cut off so many cancer sub indications could fall into that category. In the EU, the EMA sets a threshold of 5 cases per 10,000 population so around 250,000; designation is not automatic. Orphan drugs get a period of exclusive market access based on the first to approval.

A key component of this orphan exclusivity is that it is for a specific indication with a specific therapy. Thus, a CAR-T product targeting the same antigen as an approved orphan product might, in theory, be denied market access even if it worked differently and had greater efficacy and durability. However, the definition of what defines a specific orphan drug can be complex; another CAR-T with different manufacturing and efficacy/toxicity might be classed as a separate orphan product for the same condition. The EMA has revised its policy to look at not just construct or vector but manufacturing process. The EMA admits more needs to be done to ensure a careful balance between protecting innovation and promoting competition.

Novartis and Kite both have an array of orphan designations. Kymriah has orphan designation in both ALL and DLBCL in both the EU and the US, while Axi-Cel (KTE-C19) has orphan designation in DLBCL, PMBCL, ALL, MCL, CLL and FL in the US and the same indications in the EU and additionally with small lymphocytic lymphoma. Questions remain on how individual these competing CAR-T cells need to be considering they target the same antigen. Regulators as appear to be shifting away from the 'winner takes all strategy' in some of these orphan indications as they have multiple products in the pipeline showing that the orphan concessions might be too generous.[1]

One future question that is emerging is what data packages are needed for innovative T-cell therapy approvals as it is becoming apparent that it is inappropriate to follow the development

[1] The original problem was giving enough incentive to attract one company per orphan indication as major pharma companies chased mass market "blockbusters". It was then realised that a few patients with no other therapy options plus very high prices could be very lucrative.

approach of small molecules/antibodies. A late 2016 workshop at the EMA from the committee for advanced therapies highlighted future challenges.

CD19 CAR therapies for lymphomas and leukaemias

The leading CAR-T therapies target CD19, also called B-lymphocyte antigen, a type I transmembrane glycoprotein. CD19 CAR-T cells are being approved in acute lymphoblastic leukaemia (ALL) and in development in chronic lymphocytic leukaemia (CLL) and types of non-Hodgkin's lymphoma.

CD19 (cluster of differentiation 19) is an ideal target for lymphomas and leukaemias due to its expression on B-cell and its absence in other cells lines. The natural role of CD19 is to enable immature B-cells to recognise new antigens (infections) and develop antibodies against them. The new antibodies are made in quantity by mature B cells, now called plasma cells.

CD19 is expressed on both healthy and cancerous B-cells. Targeting of CD19 causes the destruction of the whole cell lineage, resulting in B-cell aplasia (over time normal B-cell recovery is possible). This does not affect memory plasma cells making antibodies against previous infections. However, patients are vulnerable to new infections till a T-cell response develops, but this takes some days. Regular gamma globulin injections are given so that they have antibody protection.

All current therapies use murine scFv to bind CD19. Use of a mouse antibody might lead to immunogenicity against the CAR-T cells over time, although this will affect long-term remission not initial response rates. Humanised scFv will probably replace some products eventually. The CD19 therapies all work slightly differently in individual and unique ways. Novartis has a follow-on humanised product to Kymriah, CTL-119.

Next developments in CD19-focused CAR-T cells

The current leading CAR-T therapies (Kymriah and Axi-Cel) utilise a murine scFv which the body could generate resistance to; resulting in decreased persistence and reduced efficacy. These can elicit a direct immune response, as with the 2013 U Penn study (NCT01355965). Initially, with preconditioning, host anti-CD19 CAR responses will be limited. In the Phase I data from the ZUMA-1 trial, no antibodies were detected against the scFv portion of Axi-Cel (KTE-C19), suggesting that at least in this product, murine antibody fragments may not be a concern. However, responses might develop in the long term as the patient's immune system recovers. This would lower long-term response rates. A trial with a CD19 CAR-T from FHCRC (Fred Hutchinson Cancer Research Center) in relapsed or refractory B-cell NHL and CLL found that a CD8+-mediated immune response was directed towards the murine scFv component. This correlated with a loss of CAR-T cells. Subsequent retreatment with CAR-T cells with or without chemotherapy in five patients led to no significant T-cell expansion or clinical response, indicating that an immune response formed. This has led to the development of human and humanised binding regions.

Novartis currently has one of the few humanised CD19 CAR-T cells in clinical development (CTL119). Data published at ASH in 2016 raised two important issues; one that humanised CAR-T cells may aid patients who are relapsed/refractory on murine CAR-T cells but equally that prior treatment with murine CAR-T cells may result in less benefit when treated with next generation humanised CAR-T cells. This is a critical question that needs to be answered: does treatment with murine CAR-T cells prevent patients from feeling the full benefit of humanised CAR-T cells in later lines of treatment?

There is an ALL Phase II University of Pennsylvania trial of a CART 19 (CTL119) due to report in H118. We anticipate that this will lead to a registration study if the data is comparable to Kymriah results. Novartis published initial data on CTL119 in May 2017 on a combination with ibrutinib in

CLL showing that eight of nine patients responded and had no detectable cancer after three months. Imbruvica (ibrutinib Jannsen), is approved to treat CLL. As a humanised product, CTL119 might be tested later in DLBCL and ALL.

Kite is also developing a humanised CAR-T against CD19. It is in trials sponsored by the US National Cancer Institute (NCI). It is being tested in two groups: patients who have never had a stem cell transplant and patents who have had an allogeneic transplant but relapsed.

Juno's JCAR014 is stated as a human antibody; it is also in an NCI study.

One outlier is a CD19 CAR NK-cell study initiated in June 2017 by Bellicum (NCT03056339) in B-cell cancers after a failed stem cell transplant. The use of a different cell linage is interesting (and may avoid IP issues), but data may not be released till 2022. The sponsor is MD Anderson showing that this is an "exploratory" or experimental study.

A further outlier is a CD22 study from Juno, JCAR018. The NCI notes that 96%-100% of most children and young adults show CD22 antigens on B-cell cancers. This fits with the FDA approval of Besponda (inotuzumab ozogamicin, Pfizer), an anti-CD22 antibody, in August 2017.

Exhibit 5: Leading CAR-T products and development candidates in CD19

Company/product/ target/partner	Disease(s)	Preconditioning	Dose	Comments on design and any efficacy data	scFv/stimulatory domains/vector/ starting cell population	Safety	Notes
Novartis/ Kymriah (tisagenlecleucel CTL-019) /CD19	Paediatric r/r B-cell ALL	r/r ALL Phase II trial: Lymphodepleting chemotherapy with cyclophosphamide and fludarabine (unspecified concentrations)	2 to 5x 10^6 CAR-T cells/kg. Maximum total dose of 2.5x10^8	ELIANA NCT02435849 ELIANA June 2017 data demonstrated that 83% (52 / 63) of patients achieved CR or CRi within three months. In addition, no minimal residual disease (MRD) - a marker of potential relapse - was detected among responding patients. Median duration of remission was not reached. The 12 month relapse free probability was 64%. Also running is NCT02228096	FMC63 (murine)/ CD3ζ & 4-1BB/ lentiviral/PBMC	In the Eliana trial 47% of experienced grade 3 or 4 CRS. 15% experienced grade 3 neurologic events; there were no Grade 4.	FDA approved 30 August 2017. 100 patient enrolment ongoing. Trial due to end 2022. Longer-term data (6 and 12 month) on remission rates is expected at major clinical meetings. Many patients received a subsequent stem cell transplant.
	DLBCL	Pre-treatments including - Bendamustine Cyclophosphamide and Radiation		JULIET study NCT02445248 June 2017 data showed ORR of 45% (23/45) with 37% CR at three month cut off.		Grade 3+ CRS occurred in 26% % Grade 3+ Neurotoxicity in 13% of patients.	130 patient study which formally ends in 2024. Data expected to be used for DLBCL NDA by late 2017/H1 2018.
CTL119 (CART 19)	ALL	NA	1-5x10^8 Split rising dosing	NCT02935543 Adult study	Humanised CAR-T		24 Patient Phase II Data April 2018
CTL119 (CART 19)	CLL	NA	1-5x10^8 Split rising dosing	NCT02640209 U Penn study backed by Novartis 8 or 9 patients fully responded so far. 15 patient study.	Humanised		Given with ibrutinib Due to complete Feb 2018
Kite Pharma/ KTE-C19 (Axicabtagene Ciloleucel [Axi-Cel])/CD19	DLBCL/TFL/ PMBCL	Concurrent cyclophosphamide (500mg/m2) and fludarabine(30mg/m3) for three days	2x10^6 anti-CD19 CAR-T cells/kg (single dose)	NCT02348216 ZUMA-1 April 2017 data was, on combined data, an ORR of 82% and CR of 54%. The median follow-up was 8.7 months with median Overall survival not reached at that time.	FMC63 (murine)/ CD3ζ & CD28/ retroviral/PBMC	Grade 3+ CRS =13%, grade 3+ neurological events = 28%, There were 2 treatment related deaths plus one other.	BLA has been submitted. Decision by late November or before.
NCI (Kite)	NHL	cyclophosphamide fludarabine	0.66x10^6 up to 18x10^6	NCT02659943 Testing with and without prior allogenic stem cell transplant,	Fully human product,		64 patients Data late 2021
Juno/JCAR-015/CD19/ Celgene (development terminated)	Adult r/r ALL	Cyclophosphamide or cyclophosphamide fludarabine	Day 1 dose of 1.0 x 10^6 CAR+ cells/kg. Dose 2 at day 28 at 3.0 x 10^6 CAR+ cells/kg	NCT02535364 halted on two separate occasions due to patient deaths. The first in July 2016 followed the death of three patients. In November 2016, Juno placed a voluntary hold on the trial after two further patient deaths.	SJ25C1 (murine)/CD3ζ & CD28/retroviral/ CD4 +CD8 co-culture	Severe CRS (Grade 3+) occurred in 27% (n=14/51) of patients, while severe neurotoxicity occurred in 29% (n=15/51).	JCAR015 was discontinued due to five fatal cases of cerebral edema
Juno/JCAR - 017/CD19/ Celgene	Lead indication is B-cell NHL. Data available in DLBCL and	NHL Phase I trial: cyclophosphamide 300mg/m2 and fludarabine 30mg/m2 for 3 days	Single dose or Two dose. Dose level 1 = 5x10^7 Cells. Dose Level 2 1x10^8 Cells	NCT02631044 Dec 2016 data: 20 efficacy evaluable patients (19 r/r DLBCL and 1 follicular lymphoma grade 3B) ORR 80% (n=16/20) and CR was 60% (n=12/20). 42% of patients (n=8/19) treated for more than 3 months showed an ongoing response. 2 patients treated at 1x10^8 cells, had a complete response.	FMC63 (murine)/ CD3ζ & 4-1BB/ lentiviral/CD4+CD8	Dose 1 - 22 patients No Grade 3+ CRS Grade 3-4 neurotoxicity in 14% (3/22) of patients. Dose 2, 3 patients none had grade severe CRS or neurotoxicity.	Defined composition (1:1 ratio of CD4+ and CD8+ cells). In NHL trial, some patients who relapsed had persistent cells. One patient had a second CR after endogenous re-expansion without a second infusion.
	paediatric ALL	Cyclophosphamide (27), cyclophosphamide/ fludarabine (14), cyclophosphamide/etopo	Dose 5x10^5 CAR-T cells/kg to 10x10^6 CAR-T cells/kg	NCT02028455 Data Dec 2016 93% (n=40/43) experienced a MRD negative CR. In patients who received cy/flu lymphodepletion, OR was 100% (14/14), estimated 12-		Severe CRS (Grade 3+) was observed in 23% (n=10/43) of patients.	

Company/product/ target/partner	Disease(s)	Preconditioning	Dose	Comments on design and any efficacy data	scFv/stimulatory domains/vector/ starting cell population	Safety	Notes
		side (1), fludarabine (1)		month event-free survival was 50.8% and OS was 69.5%			
Juno/JCAR014/CD 19	DLBCL	Cyclophosphamide and fludarabine	NA	NCT02706405	CD3ζ & 4-1BB	Stated as human antibody chain	Combination with Durvalumab (AstraZeneca CPI) given 28 days after CAR-T therapy Data Dec 2019
Juno/ JCAR018/CD22	NHL/ALL/FL Ages 1-30	Cyclophosphamide and fludarabine	NA	NCT02315612 NCI run study	NA	NA	115 patients Data Mid 2019
Bellicum CAR-T NK/CD19	B-cell cancers ALL CLL NHL	Cyclophosphamide 300mg/m^2 and fludarabine 30mg/m^2 for 3 days. Mesna is given to protect bladder from side effects	Dose escalating starting at 10x10^5	NCT03056339 .	CD28	iCasp9 suicide gene.	A different study using NK cells from umbilical cord blood which are then transformed with a CD19 CAR. 36 patient trial started in June 2017 Data June 2022.
Autolus AUTO3/ Dual : CD19 and CD22/NA	DLBCL	Cyclophosphamide and fludarabine	50 x 10^6 to 300 x 10^6	NCT03287817 (ALEXANDER) Single dose with follow-on limited duration of consolidation with anti-PD-1 (pembrolizumab) 75-day safety data and 24-month efficacy			120-patient study. Stated on website paediatric ALL also. Date March 2021. Single cell dose and 24-month follow-up.

Source: Edison Investment Research, company websites and releases, www.clinicaltrials.gov. Note: MRD = minimal residual disease (in effect a possible cure); CR = complete response; CRi = complete response but with incomplete recovery of blood cells blasts remaining; ORR = objective response rate (a response but not complete); PR = partial response. See definitions.

Kymriah CAR-T cells first to market, others to follow

Novartis gained FDA approval for Kymriah in August 2017 for paediatric and young adult r/r B-cell acute lymphoblastic leukaemia (ALL); an EMA submission will follow by the end of 2017. Novartis has also announced that it will file drug applications with both the FDA and EMA for the treatment of adults with DLBCL before the end of 2017. While in the short term Novartis will have the only CAR-T available in paediatric r/r ALL, 2018/19 could see the launch of directly competing products from Kite and Juno.

Kite Pharma has completed the rolling submission of its BLA for Axi-Cel in DLBCL also including two lymphoma variants: primary mediastinal B-cell lymphoma (PMBCL) and transformed follicular lymphoma (TFL). Axi-Cel is expected to be approved in November 2017; this has prompted the acquisition of Kite by Gilead. We expect Kite to launch at least six months before Novartis in DLBCL. As DLBCL is a larger market than ALL, the immediate constraints will be reimbursement and manufacturing capacities

Manufacturing capacity for CD19 markets may already be adequate although virus supply might limit initial sales. Kite can produce 4,000-5,000 doses per year, Novartis has a high but undisclosed potential capacity. NHL has 20,000 deaths per year in the US (SEER). DLBCL is 25% or so of NHL so the demand based on possible death rate is about 5,000 cases per year, US2.5bn at the Kymriah price of US$475,000. However, NHL is mostly diagnosed in patients over 70 and shows good responses to initial chemotherapy so this might limit CAR-T use and reimbursement. ALL is a small market on a relapsed basis. However, earlier use in the disease may be preferred and this will expand the markets considerably, there are about 72,000 NHL cases per year and 6,000 ALL.

CD19 CAR-T: Off and on target effects

Exhibit 6 (based on Exhibit 1) shows the toxicity matrix for CAR-T as it is understood currently.. Exhibit 7 shows three published early toxicity examples.

Exhibit 6: Standard CAR technology: Off and on target effects

	On target	Off target
On tumour	Has a very selective action and tumour cells are accessible for the CART-cells to detect and kill. Provided there are sufficient CAR T-cells to tumour cells, clinical "wisdom" is a ratio of 1:1 or 1:2 is ideal, as then the CAR-T efficacy can be very high. Longer-term responses are lower but impressive. Long-term survival rates are still unclear and will be complicated to interpret since many patients with CR and CRI undergo stem cell transplants.	Preconditioning regimen may mean that antigen spreading is less likely since the endogenous immune system is systematically ablated to allow room for the CART-cells to expand to the number required. Long-term persistence of CAR T-cells may be a risk.
Off tumour	CD19 CAR T-cells destroy heathy B-cells removing antibody-generating capacity (B-cell aplasia).	Not seen in CAR to date.
	Can be clinically managed by regular gamma globulin injections.	Preconditioning adds toxicity. Neurotoxicity and cytokine release syndrome effects although better managed with multiple doses.

Source: Edison Investment Research

Exhibit 7: Early serious clinical side effects in CAR-T

Target	Clinical context	Side effects	Reference
CEA	In a three-patient metastatic cancer study, CAR T-cells against CEA (found in many abdominal organs) caused regression of metastases with objective responses.	Severe transient inflammatory colitis as the T-cells attacked normal intestinal cells.	Parkhurst (2011)
ERBB2	A patient was given CAR T-cells against a well-known cancer antigen ERBB2 – the growth receptor successfully targeted by the breast cancer antibody, Herceptin.	A single high 10bn dose coupled with the fact that ERBB2 is also found in the lung (albeit at a very low level) caused a cytokine storm and fatal lung damage within 15 minutes. The patient died after five days.	Morgan (2010)
murine scFv CAR	A 2013 U Penn study (NCT01355965) gave CAR genes as mRNA requiring repeated doses every two days. Short-term expression was meant to limit long-term side effects.	One patient (of four) on the third dose experienced anaphylactic shock after five minutes with a cardiac arrest but made a full recovery. The CAR was immunogenic.	Maus et al (2013)

Source: Edison Investment Research based on cited references

CAR has the significant advantage of being able to get very high affinities against a highly specific antigen. On target efficacy with good cell expansion and persistence gives excellent response rates. Product design seems to be crucial to safety. Administration toxicities are well known if not understood and were not a regulatory concern with Kymriah. However, the favourable characteristics of the CD19 target may obscure the possibility that it is an exceptional case. In other cancers, gaining high responses with tight specificity might be harder.

Other haematological: mixed technologies

This section covers a more diverse and open area of development: multiple myeloma (MM), the biggest indication but also acute myeloid leukaemia and chronic myeloid leukaemia. The reason for the development diversity is that no antigen is the sole clear target so various antigens and technologies can potentially compete. As these are haematological indications, the cancers should be accessible to the modified T-cells so a number of these therapies could work.[2] Note that other T-cell technologies are discussed in more detail in other sections. Of these indications, MM is the biggest and perhaps another CAR-T success although CAR-T here is being challenged. AML, because if its intractable nature, offers a niche but potentially extremely valuable market if a therapy can show efficacy in relapsed and refractory patients. If T-cell therapies can offer an alternative to stem cell transplant, they could be more widely used but this depends on long-term remission rates, still unknown.

Multiple myeloma

Multiple myeloma (MM) is a cancer of plasma cells found in the bone marrow, hence the myeloid name. As MM is a white immune cell cancer, it is in effect a form of leukaemia. Plasma cells are mature B-cells that produce an antibody. Once mature, they no longer make CD19. Consequently, MM, a plasma cell cancer, cannot be treated by CD19 CAR-T therapy. Carpenter et al (2013) identified B-cell maturation antigen (BCMA, CD269), a member of the tumour necrosis factor (TNF) receptor superfamily, as a good MM target. The antigen seems very selective for MM cells. It does not seem necessary for the survival of memory B-cells or for the generation of a fresh B-cell antibody response against new infections (Tai and Anderson (2015)).

Commercially, bluebird's bb2121, an anti-BCMA CAR-T therapy targets relapsed and refractory multiple myeloma – a 50 patient Phase I trial is underway (NCT02658929). In June 2017, bluebird reported 14-day data. This was a dose escalation phase. Of 15 patients treated who received $150x10^6$ cells or more (top dose $800x10^6$), 100% had an objective response. Of these, 26% (4/15) were deemed complete responses. Most (71%) had CRS but mostly grades 1 and 2. This data is very strong given the advanced nature of the disease. Cohort expansion started in September 2017 with completion in late 2018.

Also targeting BCMA with CAR-T is Juno with an academic-lead MM study. The dose escalation might complete in 2020.

Autolus has a new MM study with its dual CAR-T cell therapy that has two CARs, one binding BCMA and the other the transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI). The idea of this complexity is to prevent antigen escape and target the tumour more effectively as some cancer cells may have low BCMA expression. It is in dose-ranging studies.

GSK is developing a monoclonal antibody against BCMA. It is in a dose escalation study followed by possible cohort expansion (NCT02064387). Data is expected from late 2018, if released.

[2] Bone marrow and lymph tissues are accessible to T-cells while entry into solid cancers, discussed later, is much harder. Cancer cells in the circulation should be easily eliminated if the antigen targeting is correct.

Celyad is running a clinical trial in NKR-CAR T-cell therapy in MM and AML. This trial, part of the THINK study, is still in dose escalation, up to $3x10^9$ cells are given per dose with three doses administered given every two weeks. A 14-patient per cancer cohort expansion is planned in both indications. NKR therapy is covered in detail below.

Adaptimmune started an MM trial (NCT01352286) in August 2012 using a TCR against the well-known internal cancer-testis antigen, NY-ESO-1. This is found in about 60% of MM cases. This project is partnered with GSK. The patients all received autologous stem cell transplants with TCR therapy given two days later. The rationale is that autologous grafts are poor at attacking any residual MM cells as they are still seen as "self" whereas allogenic stem cell transplants are much more efficient at elimination; however, allogeneic grafts are not always possible. The aim was to see if an allogeneic type response could be generated by autologous TCR cells.

Recruitment completed in 2015. Results reported by Rapaport et al (2015) noted encouraging clinical responses in 16 out of 20 patients with TCR cell expansion and persistence. Lack of persistence was associated with disease progression. In October 2016, an agreement was reached with Merck to test this TCR therapy in combination with Keytruda, the leading PD1 inhibitor. This trial is still at the pre-IND stage.

Acute and chronic myeloid leukaemias (AML and CML)

This is a diverse group of cancer subtypes derived from a myeloid cell line, that is the cells would, if healthy, develop into other immune white cell types like natural killer cells or macrophages (but not B or T-cells), or blood cells like platelets or red blood cells. Accordingly, AML and CML do not carry CD19 or BCMA. The cancer cells move from bone marrow into the blood so are a leukaemia.

AML is an intractable condition treated with stem cell transplantation if there is a good response to first line chemotherapy. If not, various rescue therapies are tried - but most do not succeed. As an acute condition, disease progression can be rapid. Patients often move through a series of clinical studies as AML is a popular therapeutic target. AML is a hard to manage condition so any survival gains from T-cell therapies will be welcome.

Bellicum has started an AML trial (NCT02743611) with a TCR targeting the PRAME[3] antigen. Data is due by late 2019 but as open label trial, earlier results may be posted. PRAME was detected in melanoma but is widely found in a broad range of solid cancer indications. Any success in AML could lead to other, larger, indications being developed.

There are a number of academic studies using CD30 CAR-T. Only Ziopharm has a CD33 CAR-T in an exploratory clinical trial led by M.D. Anderson. CD33 is a marker for myeloid cells but also occurs on lymphoid tissues so is not fully specific. CD33 is also targeted by the therapeutic antibody, Mylotarg (gemtuzumab ozogamicin, Pfizer), recently re-approved by the FDA.

In AML, Celyad has a dose ranging study with its NKR-CAR T-cell therapy. One AML patient at low dose in an earlier study showed an unexpected stable disease response. More data is awaited,

Cellectis is testing a CD123 allogeneic CAR-T cell (UCART123). This is on hold due to toxicities. There is also a small exploratory academic study with a CD30 CAR-T.

Juno has an academic-sponsored TCR study with JTCR016. This study was suspended in October 2016 although it may restart. It began recruitment in 2012. It targets Wilms Tumour antigen (WTI).

CML progresses slowly. It is a much smaller indication than AML and is well managed. Some 95% of CML cases have a chromosome change (Philadelphia translocation) that produces a new enzyme. This is inhibited by Gleevec (imatinib), now generic, Bosutinib (Bosulif) Sprycel (dasatinib)

[3] PRAME = PReferentially expressed Antigen in MElanoma.

and Tasigna (nilotinib). No T-cell therapies are being developed for this indication as yet but exploratory T-cell trials might be run once other indications are approved.

Other haematological (non CD19): Off and on target effects

Exhibit 8 shows the toxicity matrix for non CD19 CAR-T in haematological cancers. Given the much earlier clinical status and mix of technologies being tested, this picture is less clear.

Exhibit 8: T-cell technologies in other haematological cancers – off and on target effects

	On target	Off target
On tumour	BCMA – appears very selective with good response rates in initial bluebird bb2121 data.	Preconditioning regimen, if used, may mean that antigen spreading is less likely,
	CD123 – allogeneic approach, no clinical data	Too little information to be certain.
	NKR-CAR – limited data as yet; more expected by 2018.	
	TCR – early trial showed sustained responses but extent to which residual disease eliminated might be limited. Combination therapy with Keytruda being explored.	
Off tumour	BCMA might lead to some reduction in immune function but no data released. Any loss of antibody production can be clinically managed by regular gamma globulin injections.	To date, not seen in any therapy but limited data. Preconditioning adds toxicity if used.

Source: Edison Investment Research

Exhibit 9 lists leading western industry-led or co-sponsored studies. There are also a number of Chinese clinical trials in this area, not shown.

Haematological conclusions: many unresolved issues

In end of line severely ill patients, acceptable levels of safety are lower as patients look to all and any treatments that often only offer limited extensions in either the quality or duration of life. CAR-T treatments offer a tantalising opportunity to potentially cure patients. However, achieving this is fraught with complex, numerous and potentially deadly complications.

Control of toxicities will be key to the future of CD19 CAR-T products and their ability to move beyond second- and third-line treatments. CRS appears to be manageable and a consequence of CAR-T efficacy. Neurotoxicity remains difficult to manage and control. Long-term safety has yet to be concluded: the impact of persistent engineered chimeric T-cells could lead to a permanent change in the immune systems (eg graft versus host disease with allogenic CAR-T cells, B-cell aplasia).

Any successful move towards earlier lines of treatment and larger accessible patient populations is dependent on the ability to minimise these toxicities. The appropriate use of lymphodepletion, control of expansion, if possible, addition of control genes and modification of overall construct design will all prove pivotal in determining the success of any CAR-T products. The ability to move away from lymphodepletion would prove advantageous – but may not be possible.

In other haematological cancers, it is likely on initial Bluebird bb2121 data that MM will also become a major T-cell therapy market as chemotherapies eventually lose control of the disease in many patients. BCMA is in the lead but other technologies like NKR CAR T-cell might be as effective and/or cheaper. MM might be a more complex market than CD19 with contrasting products.

AML is very intractable but if a T-cell therapy enabled rescue of relapsed and refractory AML patients, or just gave prolonged remission, it would have a dominant position. Note that AML is a complex set of sub types so only some might respond well.

The ability of manufacturers to supply sufficient quality of T-cell therapies in significant quantities remains to be seen at a commercial level. This applies to virus for production (a serious current bottleneck) as well as the more visible cell manipulation and testing done by companies. Currently,

between 10% and 20% of patients die between enrolment and CAR-T infusion. Current centralised manufacturing times of between two to four weeks need to be shorter. Allogeneic products, if they work, could be stored at some major centres for immediate use. Allogeneic may be the key to a mass market for CAR-T.

Exhibit 9: Leading T-cell and development candidates in other (non-CD19) haematological cancers

Company/Product /Target/Partner	Disease(s)	Preconditioning	Dose	Most advanced trial data (efficacy)	scFv/stimulatory domains	Safety	Notes
Bluebird/bb 2121/BCMA/CAR-T/Celgene	r/r MM	Fludarabine (30mg/m2) and cyclophosphamide (300mg/m2) for 3 days	data on 4 revealed. $5x10^7$, $15x10^7$, $45x10^7$ $80x10^7$ $120x10^7$ (planned)	NCT02658929 In 15 patients receiving $15x10^7$- $80x10^7$ CAR-T cells, four had complete responses (27%) and all had an objective response (complete, very good or partial). There is no obvious dose-response relationship so far. However, numbers are small and these were heavily pre-treated patients who have failed stem cell transplants and relapsed and refractory disease.	BCMA scFv, CD3ζ 4-1BB, lentiviral	CRS in 71% of patients, mostly grade 1 or 2. No dose limiting toxicities to date	50-patient study. Data due December 2018
Celyad NKR-CAR T-cell	MM AML	No preconditioning so little cell expansion seen	$3x10^8$ $1x10^9$ $3x10^9$	NCT03018405 Three doses of cells given two weeks apart. Dose escalation study with 14 patient cohort expansion phase planned in MM and AML at highest dose reached.	NKG2D/DAP10	No events reported to date	One AML patient in previous study had unexpected stable disease. Dose data late 2017/H1 2018.
Cellectis CAR-T	AML	Used but not stated	$6.25x10^5$/kg to $6.25x10^6$/kg	NCT03190278 **On hold**. 156 patient dose escalation study. Single iv dose. Dose expansion Phase II cohorts, r/r and first line.	UCART123/NA	Severe side effects noted and one fatality.	Allogeneic constriction targeting CD123. Completion late 2021 (?)
	MM	Assumed use planned	NA	Preclinical.	UCART38	NA	Planned development
Intrexon/Ziopharm/ CD33 CAR-T	AML	Fludarabine and cyclophosphamide	$1.5x10^5$/kg to $4.5x10^5$/kg	NCT03126864 Adult and paediatric arms. 39 patient trial.	CD33/NA	NA	Run by MD Anderson. Data H221.
Juno/BCMA/ CAR-T	MM	Cyclophosphamide Lenalidomide.	$1x10^6$, $3x10^6$, and $1x10^7$	NCT03070327 Dose ranging	4-1BB	NA	Q1 2020 data, 36 patients
Juno/WTI/TCR JCTR016	AML	Fludarabine and cyclophosphamide		NCT01640301 Academic sponsored	NA	Suspended recruitment Oct 2016. started 2012	In patients who have failed stem cell transplants.
Adaptimmune/ NY-ESO-1 TCR	MM	Not used as given 2 days after autologous stem cell transplant	$2.4x10^9$ cells	NCT01352286 Encouraging clinical responses were seen in 16 out of 20 patients with TCR cell expansion and persistence. A median progression free survival of 19.1 months was reported.	NA	No CRS although high IL-6 levels seen	A second study in combination with Keytruda is being planned.
Bellicum/ PRAME TCR	AML	Not stated but lymphopenia required to allow expansion	Dose escalation	NCT02743611 A 40-patient study. The TCR construct includes the rimiducid suicide switch in case of adverse reactions or GvHD.	NA	NA	Results due late 2019.
Autolus/AUTO3) Dual :BCMA and TACI	MM	Fludarabine and cyclophosphamide	15×10^6 to 350×10^6 Single or split dose	NCT03287804 (APRIL)	NA	NA	80-patient study, dose ranging then expansion. Data October 2020 after 12-month follow-up.

Source: Edison Investment Research, company websites and www.clinicaltrials.gov.

Section 2: CAR-T in solid cancers

The leading "standard" CAR-T players seem to be very focused on haematological developments - as expected. Novartis and Bluebird have no visible clinical stage projects in solid tumours. Kite has no CAR-T in solid cancers but has an exploratory TCR study.

Bellicum has one CAR T-cell solid tumour project and one TCR project.[4] The CAR T-cell lead indication is in pancreatic cancer using prostate stem cell antigen (PSCA). PSCA is expressed on several solid tumour cancer types so this could be utilised in multiple indications. Bellicum uses GoCART technology (see above) to control the CAR-T cell response according to the concentration of rimiducid (a small, non-drug molecule) and therefore aims to limit any side effects.

Juno is running three CAR solid cancer studies (as listed on the company website) plus a TCR study (JTCR016, see TCR section). An academic, Juno co-sponsored trial is testing the "armoured CAR" approach in sold cancers. This uses a CAR-T therapy (JCAR20) targeting an MUC16 antigen and enhanced to express the cytokine IL12 to trigger a systemic immune response. This is due to produce data in H218. There are two other solid cancer trials, but with academic sponsors so these appear exploratory. One is a neuroblastoma trial using JCAR023 targeting L1CAM and the other uses JCAR024 to target ROR-1 for nsclc, breast and various haematological indications.

Unum, a new entrant, has a different approach whereby the CAR binds (using CD16) the Fc region of existing therapeutic monoclonal anti-cancer products like rituximab. Rituximab targets CD20 found on immune cells. The CAR-T-cells therefore only attack tumour cells marked by the therapeutic antibody. This strategy depends on there being an effective and selective enough approved therapeutic antibody. Progress seems to have been limited.

Antigens used

Using the cliniclatrials.org database plus company websites, we have compiled a table which shows current solid tumour CAR trials. The pattern is of small scale studies, the largest being a 107 person NCI trial in glioblastoma (NCT01454596) targeting a mutated growth factor (EGF variant 3); this is a classic dose escalation followed by expansion study. Generally, the concerns are about safety at this stage. The larger studies tend to be testing several cancer types. Primary completion dates are shown as in cliniclatrials.org but experience indicates that these often slip as patient by patient safety studies tend to take longer due to recruitment issues. Academic studies are also slower usually than commercial ones; reporting of studies may take some time.

Cheever et al (2009) scored cancer antigens but interestingly, few of these have made the CAR clinical selection in western clinical trials so far, Exhibit 10. Note, this is a mix of commercial and leading academic studies but not all academic studies are cited. The University of Pennsylvania is linked to Novartis and MSKCC has connections to Juno.

The pattern seen is of commercial caution. Only Bellicum has an outright trial and that is exploring the use of its CID technology designed to control the level of CAR activation though rimiducid activation of an inducible MyD88 co-stimulatory domain. The ROR1 study linked to Juno is evaluating both solid and haematological cancers. Juno is only named as a collaborator on the MUC16 targeted armoured CAR study sponsored by MSKCC.

[4] There is also a CAR NK cell project (NCT03056339) in B-cell malignancies.

Exhibit 10: Standard CAR T-cell target antigens in US trials

Antigen (NCI score)	Cancer type	Company /academic	Trials	Size	Data due	Comments
EGFR variant 3 (0.76)	glioblastoma	U Penn	NCT02209376	12	Q3 2018	Pilot study
		National Cancer Institute	NCT01454596	107	Q4 2018	Single arm study with dose ranging followed by cohort expansion at MTD.
MUC16	Solid tumours JCAR020	MSKCC / Juno	NCT02498912	30	Q3 2018	"armoured" CAR T-cells :secretion of IL-12
L1 CAM (CD171) (NA)	Neuroblastoma, JCAR023	Seattle Children's Hospital (Juno link)	NCT02311621	40	Q4 2017	Toxicity 28 day study, CD171 has wide neural expression
CEA (0.62)	Liver metastases	Roger Williams Medical Center	NCT02850536 NCT02416466	5 8	Q4 2017 Q3 2017	Three doses Plus Y-90 beads (Sirtex)
Mesothelin (0.41)	Pancreatic, Ovarian and mesothelioma	U Penn	NCT02159716	19	Q4 2015	Uses 4-1BB ongoing not recruiting.
			NCT03054298	30	Q1 2021	Human CAR, with and without preconditioning.
	Malignant pleural (lung) disease and breast	MSKCC	NCT02414269	24	Q2 2018	Safety and biomarker efficacy
ROR1+ (NA)	non-small cell lung cancer, or triple negative breast	FHMRC (Juno link)	NCT02706392	(60)	Q4 2021	JCAR024 NB Has haematological and solid cancer arms,
PSCA (0.41)	Pancreatic, BPX-601	Bellicum	NCT02744287	30	Q4 2020	Uses a controllable co-stimulatory domain activated by rimiducid

Source: Edison Investment Research based on cited studies. Note: ROR1+ = receptor tyrosine kinase-like orphan receptor 1 positive; PSCA = prostate stem cell antigen; NCI = National Cancer Institute; MSKCC = Memorial Sloan Kettering Cancer Center; FHCRC = Fred Hutchinson Cancer Research Center; NCI score for antigen use based on Cheever et al (2009).

The other major observation is the involvement of Chinese hospitals and companies in solid tumour CAR development, Exhibit 11. A wide range of antigens is being tested with a focus on liver, brain, lung and GI cancers. This is interesting not because these constructs will quickly gain western clinical approvals, they might eventually, but rather that the data could show if any of these antigens are suitable targets for the US leaders to pursue and could indicate the side effect profiles.

Exhibit 11: Standard CAR-T therapies target solid cancer antigens in Chinese trials

Antigen (NCI score)	Cancer type	Company /academic	Trials	Size	Data due	Comments
EGFRv3 (0.76)	Glioblastoma	Marino Biotech	NCT02844062	20	Q3 2019	
MUC1 (0.79)	Nsclc, pancreatic triple neg breast, liver, colorectal, gastric, brain	PersonGen BioTherapeutics	NCT02587689 NCT02617134 NCT02839954	20 20 10	Q4 2018 Q4 2018 Q3 2018	
CEA (0.62)	Solid tumours, breast, gastric, colorectal, pancreatic, lung	Southwest Hospital	NCT02349724	75	Q4 2019	local delivery via catheter
	Liver	Shanghai GeneChem	NCT02862704	20	Q2 2017	local delivery via catheter
glypican-3 GPC3	Liver	Shanghai GeneChem	NCT02715362	30	Q3 2018	local delivery via catheter
	lung	Carsgen	NCT02876978	20	Q2 2019	
EpCAM (0.48)	Gastric	Sinobioway Cell Therapy	NCT02725125	19	Q4 2019	Epithelial cell adhesion molecule is expressed on tumour cells but can be lost as tumours progress.
	Liver		NCT02729493	25	Q4 2019	
GD2 (0.65)	Paediatric Neuroblastoma		NCT02919046	22	Q3 2020	
Mesothelin (0.41)	Solid cancers	Ningbo Cancer Hospital	NCT03030001	40	Q4 2018	Combines a PD1 checkpoint antibody and CAR
	Pancreatic, Ovarian and mesothelioma	China Meitan Hospital	NCT02930993	20	Q3 2018	
	Pancreatic	Shanghai GeneChem	NCT02706782	30	Q1 2018	local delivery

Source: Edison Investment Research based on cited studies April 2017. NCI score for antigen use based on Cheever et al (2009).

CAR: Off and on target effects in solid cancers

Exhibit 12 shows the toxicity matrix for CAR as it is understood at present. CAR has the big advantage of being able to get very high affinities. The other toxicities are well known if not understood. On target efficacy with good cell expansion and persistence could give excellent response rates.

However, in solid cancer, the on target, off tumour side effects could be significant and will limit progress. They may be overcome in specific cancers; progress in brain cancers would be wonderful, but the antigens may not be specific enough.

Exhibit 12: Standard CAR technology – off and on target effects

	On target	Off target
On tumour	It is not certain, and probably very unlikely, that CD19 CR can be replicated in solid tumours given that solid tumour cells are heterogeneous and embedded in matrix and a more sustained, broad spectrum immune response may be needed.	Preconditioning regimen mean that essential antigen spreading is unlikely. Use of IL12 in "armoured" CAR is an attempt to gain additional immune support. Antigen spreading may also be achieved by the use of PD1 and CTLA-4 COPIs in combination.
Off tumour	Lack of specific solid tumour antigens means that these toxicities have been observed in earlier studies and are likely to occur at some level.	Preconditioning adds toxicities.
	Various "tuneable" and suicide technologies being tested (Unun, Bellicum). Data still lacking.	CAR-T specificity can be made very high so this should be avoidable.

Source: Edison Investment Research

CAR-T: The right way forward in solid cancer?

Of the studies in Exhibits 9 and 10, four are by two western companies with the resources to take a product through FDA approval. The rest are either relevant academic studies that could develop into commercial indications, or Chinese studies included to show that data from different antigens will become available, assuming the trials are fully published. Of the US companies, Bellicum is using its rimiducid–based tuneable technology to control potential side effects. Juno is evaluating its "armoured" CAR to boost efficacy by producing the cytokine IL12. The impression is that the US companies are very focused on haematological cancers and still view solid tumours as intractable and very risky territory for standard CAR-T technology. The next Section 3 looks at a different, more generalist approach to solid and haematological cancers in Natural Killer Receptor T-cell technology. As this is not accessible to standard CAR-T companies, some are experimenting with TCR T-cell therapy, see Section 4.

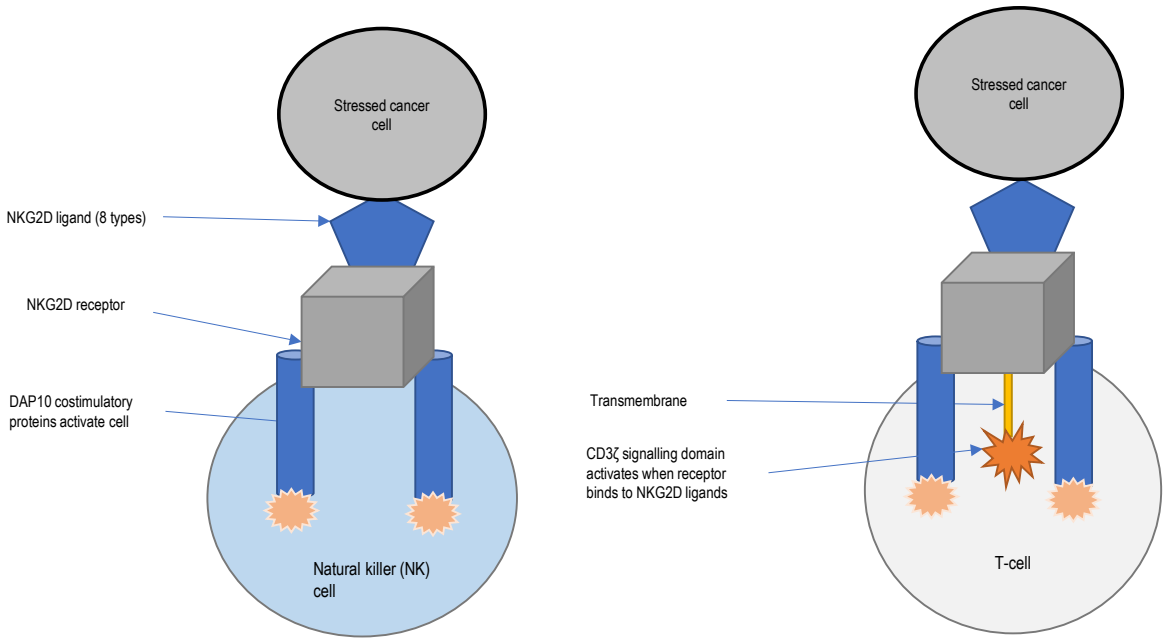
Section 3: NKR T-cell therapy Natural born killers

Celyad, protected by intellectual property, controls the natural killer, NKR CAR T-cell area. It is developing CYAD-01 as the lead product. This section summarises key background data on the NKR CAR technology to allow comparison with other T-cell approaches. The basic work on NKG2D CAR, including filing of key patents, was done by the group led by Professor Sentman at Dartmouth College (US), Sentman and Meehan (2015). See Demoulin et al (2017) for a recent review.

NKR technology

This technology takes a natural killer cell receptor, modifies it and inserts it into killer T-cells to make an NKR CAR T-cell therapy, Exhibit 13.

Exhibit 13: Natural killer receptor CAR T-cell technology



Source: Edison Investment Research

In endogenous NK cells, the NKG2D receptor does not have a CD3ζ and all signalling is done by DAP10. DAP10 proteins bind and activate the PI3K signalling cascade so activating AKT (Protein Kinase B) and mTOR, powerful interconnected internal cell signalling molecules. PI3K enhances cell survival and growth. Hence, the Celyad NKR CAR construct has a powerful activation effect as it adds CD3ζ activation of a different signalling though ZAP10 (not shown).

The activated complex has six molecules, all of which provide an activation signal. Only this hexameric complex is stable on the cell surface. The set of genes for the NKG2D CAR construct is inserted into autologous T-cells using a retroviral vector.[5]

Killer T-cells naturally express NKG2D and DAP10 as co-stimulatory mechanisms. Normal NKG2D receptors are not sufficient to activate a T-cell without TCR activation as well. In nature, NKG2D modulates the cytokine release pattern of the T-cell (Barber and Sentman (2011)).

[5] Vector: Moloney Murine Leukemia Virus (Mo-MuLV)-based oncoretroviral vector SFG-chNKG2D.

Packaging cell line: PG13. Vector particles are pseudotyped with the Gibbon Ape Leukemia Virus (GAL-V) envelope glycoprotein (source Celyad 2016 R&D presentation).

NK cells are not an efficient cell therapy being difficult to culture. The NK cells are also inhibited from attack if the target cell has a high enough level of MHCI, the HLA self-antigen system. This is why the chimeric NKG2D receptor is inserted into T-cells in the Celyad approach.

NKG2D ligands

In contrast to standard CAR, where an antigen has to be selected and a synthetic antibody engineered, NKG2D has a naturally high affinity (about ten times other immune ligands[6]) with an evolved suite of eight natural human ligands. The mouse NKG2D system is very similar so the preclinical models of this system are believed to be a good guide to the potential clinical situation.

The discovery of these ligands is reviewed by Champsaur and Lanier (2006). The main ones studies are MICA and MICB proteins.[7] There are also six members of the UL16-binding[8] protein family: ULBP1-6. Spear et al (2013)) collated data from a range of literature showing widespread expression of NKG2D ligands in most solid tumours and also in leukaemias, lymphomas and myeloid cancers. In normal human tissues, the NK ligands are only found in the intestine (Groh et al (1996)); the evidence for more widespread prevalence of NK-ligands in healthy tissues was reviewed by Eagle et al (2009)..

Exhibit 14 shows evidence for NK ligand expression on solid tumours involved in the current Celyad THINK clinical trials. Studies using PCR as a method to detect NK ligand mRNA might overestimate the cell surface expression as mRNA for MICA, MICB and ULBP does not directly correlate with cell surface expression. There have been only limited comprehensive clinical studies on NK ligands as clinical markers of prognosis. Exhibit 14 cites studies we have found that use antibody data as this is more relevant than genetic and transcription data.

Exhibit 14: Clinical observations on NK ligand expression in cancer

Cancer	Selected observations on ligand incidence
Ovarian	For example, Li et al (2009) found MICA or MICB in 97.6% of ovarian cancer cells and ULBP2 in 82.9%; neither was expressed on normal ovarian epithelium. The expression of MICA/B was highly correlated with ULBP2. Strong expression of ULBP2 in ovarian cancer cells was correlated with less intraepithelial infiltration of T-cells and bad prognoses for patients.
Colorectal	Watson et al (2006) found that MICA expression was correlated with better survival in 449 colorectal cancer patients.
Pancreatic	Duan et al (2011) MICA was detected in 89.3% of pancreatic cancer tissues (100 samples) at higher levels than were in normal pancreatic tissues. Soluble MIICA levels gave a worse prognosis.
Breast	Madjd et al (2007) in 530 breast cancer samples found upregulation of MICA in poor prognosis grade 3 tumours. In over 50% of patients, 75% or more of the cells showed MICA expression although intensity of MICA expression was variable.
Bladder	Ferreira-Teixeira (2016) found that adoptive NK cells from patients activated by cytokines were active against cultured bladder cancer stem-like cells which expressed MICA/B and ULBP ligands amongst others.

Source: Edison Investment Research

One oddity in Exhibit 14 is that high NK ligand levels are a poor prognostic indicator in ovarian cancer but a good one in colorectal. This might be because Watson et al (2006) measured colorectal survival from the date of surgical resection of the tumour - which might have broken immune tolerance. Duan et al (2011) noted NKG2D upregulation in pancreatic cancer after resection. Okita et al (2016) in cisplatin treated nsclc after resection found that upregulated MICA was indicative of a good prognosis – cisplatin upregulates NK ligands, see below.

Possibly, as Li et al (2009) found in ovarian cancer, high MICA levels on diagnosis probably means lack of NK immune surveillance as these cells were not being attacked. Madjd et al 2007 found that there was a lack of NK cells in breast cancer tumour biopsies despite high MICA expression so the cells were tolerating the tumour.

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6 The affinity is naturally roughly ten-fold higher, in the range about 0.0 to 1 μM, than many other immunoreceptor-ligand interactions. For example, the inhibitory receptor CTLA-4 binds to its ligand CD80 with an affinity of 0.4 μM.

7 MHC class-I-chain-related protein A (MICA) and protein B (MICB).

8 UL16 is a herpes viral protein and nothing to do with cancer and the NKG2D system. The name is historically related to the discovery of the protein.

Production of NKG2D ligands by genetically damaged cells

NKG2D ligands appear to be produced and are at a maximum after 24 hours of DNA (genetic) damage (Gasser et al (2005)) or infection. This is due to tightly-regulated DNA damage response and repair system linked to the ATM (Ataxia telangiectasia, mutated) or ATR (ATM- and Rad3-related) internal cell signalling kinase systems. The NKG2D ligands are produced as an external signal as part of this response.

DNA damage agents like 5-fluorouracil (5FU) or cisplatin also upregulate these ligands. Proteasome inhibitors like Velcade (bortezomib indicated for multiple myeloma) at low doses increases NK ligand cell surface expression and NK cell killing of cancer cells (Niu et al (2017)) - perhaps by reducing degradation of these ligands inside the cell and so aid surface expression. HDAC inhibitors like valproate (an anti-epileptic agent) increased NK-ligand expression (Armeanu et al (2005)). These could be possible future drug combinations with NKR CAR T-cell therapies.

The science is a little more complex however; more detailed investigation (Iannello and Raulet (2013) shows that normal cells constantly transcribe the NK ligands but do not translate them into proteins.[9] Those NK ligands that are made are quickly marked for destruction so very few appear on the cell surface. However, if the cell becomes genetically damaged or stressed, then the blocks on producing protein are removed; more protein is made and these are less likely to be destroyed so the expression on the cells surface rises quickly.

Importantly for therapeutic use, this stress response is not universal, that is, many of the factors that often stress healthy cells like lack of oxygen, too much acid or higher temperatures did not cause NKG2D ligands to be produced. This is important for potential toxicity of the NKR T-cell therapy as it minimises the risk of on target, off tumour toxicities. Champsaur and Lanier (2006) commented that, “In general, there is consensus that if NKG2D ligands are expressed in normal adult tissues, it is in low amounts, possibly below the levels needed to activate immune cells expressing NKG2D receptors.” If NKR T-cells are given with chemotherapy, normal tissues may also experience genetic damage so a wash out period would be indicated, as in the proposed SHRINK CYAD-01 study in combination with 5FU in colorectal cancer.

An exception is oxidative damage due to abrupt switches between a hypoxic (oxygen deprived) and reperfused state. Normal endothelial cells exposed to H_2O_2 express NK ligands due to sudden oxidative damage. This might be why tumour vasculature, which is poorly formed and convoluted, expresses NK ligands and so can be attacked by NKR T-cells (Zhang and Sentman (2013)). This anti-angiogenic effect could be very important therapeutically.

CYAD-01 clinical trials

The current THINK (THerapeutic Immunotherapy with NKR) trial is in two arms: solid and haematological. Trial details for solid tumours are in Exhibit 15; the two haematological cancers were discussed in Section 2, Exhibit 9.

THINK follows the haematological single-dose escalation study (in AML and MM) that reported in December 2016 and showed safety up to $30x10^7$ cells. There was one unexpected report of efficacy in an AML patient at the highest dose. This patient showed stable disease over at least six months.

[9] This appears to be regulated by microRNA by destroying the mRNA.

Exhibit 15: NKR CAR T-cell solid trials

Antigen	Cancer type	Company	Trials	Size	Data due	Comments
MICA MICB ULBP1-6	Multiple dose ranging, three given 14 days apart over 28 days • $3x10^9$ • $1x10^9$ • $3x10^9$	Celyad	THINK study Clinicaltrials.org NCT03018405 EudraCT 2016-003312-12	12	H2 2017 8	Three per cohort, any eligible cancer type. Initial cohort recruited colorectal and pancreatic. At highest tolerated dose, a further three patients will be recruited giving six in the MTD cohort. In the event of toxicity, a further patient is added to the cohort for further evaluation.
	Ovarian			14	Q4 2018 Q2 2019 Q2 2020	6 month follow-up data 12 month follow-up data 24 month follow up data (primary endpoint) Patients in the cancer type entered in the highest dose ranging level will move automatically to the appropriate trial arm.
	Triple negative breast			14		
	Colorectal			14		
	Pancreatic			14		
	Bladder			14		
	Colorectal in combination with chemotherapy		SHRINK	NA	NA	Q217 expected start. Chemotherapy will be FOLFOX (or FOLRI) both using 5FU known to potentiate NK ligand expression. NKR T-cells probably given 3 days after chemotherapy for safety reasons.
	Colorectal with direct intra tumour injection		LEAP	NA	NA	Q317 expected start.

Source: Edison Investment Research. Note: Similar studies are running in AML and MM in a parallel THINK trial arm.

The THINK study dose-ranging element tests three doses per level administered every 14 days over a 28-day period. CD19 CAR-T studies showed that multiple dosing reduces side effects like cytokine release syndrome; CRS has not been seen in CYAD-01 therapy to date. The other factor behind repeat dosing is that the NKR CAR T-cells have a short persistence. After infusion into mice, the cell number peaks at day three and drops by about half by day 7.[10] CYAD-01 cells can be found in the bone marrow and spleen of mice at low levels after infusion. They show persistence of less than seven days (Barber et al (2011)). This could be a major safety advantage as it reduces the on target off tumour toxicity risk if patients get a viral infection or sepsis.

In THINK, patients can be diagnosed with any of the five eligible solid cancer types. This part of the trial is a dose escalation study so uses staggered enrolment: new patients wait till the previous patient has received their second dose; this is a safety feature. The highest dose is assumed to be $3x10^9$ but this will be adapted for responses seen. Once a dose level is established in a three patient cohort, then a further three patients are recruited to confirm this. The trial then moves to the expansion phase to test efficacy. If toxicity is seen, a further patient is tested at that dose to confirm the observation.

The expansion phase plans to enrol up 64 more solid tumour patients. This enables each indication to be evaluated independently. Combined with the six patients in the highest dose phase, this is meant to give 14 patients per indication. In this design, at least one objective response per indication in the first seven patients is expected as an overall indication of potential efficacy. Any tumour types that do not achieve this should terminate, but this depends on what responses are seen: CYAD-01 therapy is not standard chemotherapy and prolonged stable disease responses in solid cancer could be clinically valuable. Chemotherapy only works if there is rapid tumour ablation.

Celyad has considered the possibility of extending promising indications into larger Phase II studies for accelerated approval. This could enable a fast route to market. If so, manufacturing will need to be scaled up quickly. The NKR technology has the advantage of a single vector used for multiple cancer types. Vector supply will also need to be boosted as this is sourced from third parties.

[10] Slide 80 (page 78) Celyad 2016 R&D day presentation. Reference Spear and Sentman unknown so appears to be unpublished material.

Toxicity estimates and preconditioning

There remains a potential concern with NKR T-cell therapy about on target, off tumour toxicity with the intestinal lining as a possible site.[11] In a detailed paper, Sentman et al (2016) examined NKR T-cell therapy toxicity.

- In a mouse model, single doses of 20 million cells were toxic with cytokine release-like symptoms driven by GM-CSF release but not affected by IFNγ. An intact host immune system was needed for the effect.
- A dose of 10m cells was not toxic although there seemed to be minor initial effects on cell infusion that quickly resolved.
- Giving multiple doses gave no additional toxicity.

Scaling this up, a mouse weights about 20g so 20m cells is $1x10^9$/kg. The average US male weighs 195lb (88.4kg), the average US woman 166lb (75.2kg) (source CDC). This averages about 82kg. On the same dose per kg as a mouse, the toxic NKR T-cell dose would be $83x10^9$ (82 billion) cells and the safe dose about 40 billion cells in a single administration. The highest proposed single dose is $3x10^9$ in the THINK study so the implication, yet to be proven, is that the higher THINK dose is about 27-fold lower than the toxic dose in mice. Direct comparison is not reliable as there are other factors but on the available evidence, NKR T-cell therapy should have a wide therapeutic window assuming that efficacy is seen at $3x10^9$ cells or lower.

This raises the aspect of preconditioning or lymphodepletion. In standard CD19 CAR-T therapies, and also in TCR T-cell approaches, preconditioning depletes the host immune system. It helps remove Tregs that might limit a CAR therapeutic effect, acts on the tumour and perhaps most importantly allows the rapid expansion perhaps 1000-fold or more, of the number of infused CAR T-cells. It is this rapid expansion coupled with the extent of the tumour being attacked that causes cytokine release syndrome. CAR approaches now give lower doses over several days to limit this. In a preclinical study of CYAD-01 in multiple myeloma (Barber (2011)), the Sentman group tested lymphodepletion with cyclophosphamide and showed that this had no effect on overall survival.

Resistance to NKR-targeted attacks by tumours

Tumours are dynamic living systems that evolve under selection pressure. The pattern of expression in a tumour is likely to be heterogeneous and not all cancer cells need to show NKG2D ligands to trigger a generalised immune attack. One of the key features of the NKR T-approach is that the endogenous immune system (T-cells and NK cells plus macrophages) remains intact and is needed to participate in an anti-tumour response.

There is a literature report by Friese *et al* (2003) that glioblastoma (brain cancer) resist attack by NK cells due to higher MHC I expression. However, increased NKG2D stimulation overcame this in animal models [12] and gave lasting immunity; it is possible that an NKR CAR T-cell might have a similar effect. NK cells are inhibited by self MHC I on cells - unless the level of NKG2D ligand binding is high enough to overcome the inhibition and then the NK cell attacks.

Cancer cells might also downregulate NKG2D ligands or affect NK and T-cells though cytokines and secretion of TGFβ (see section LINK) under selection pressure from the immune system. As examples, Mamessier *et al* (2011) found that breast cancer could induce tolerance to NK surveillance. There was a high level of ligand shedding involved as well – soluble ligands released by cancer cells bind and block the NK receptors (Groh et al (2002)) at a concentration of 100 ng/ml. The level of soluble antigens has been cited as a market of poor prognosis but Holdenrieder *et al*

[11] The NK ligand expression is not uniform and restricted to the upper villi epithelium and does not involve the crypts. Hence, even if some GI effects occur, they should be short lived as the upper villi epithelium is regenerated from the cells in crypts over a few days.

[12] The researcher used a virus to boost MICA levels.

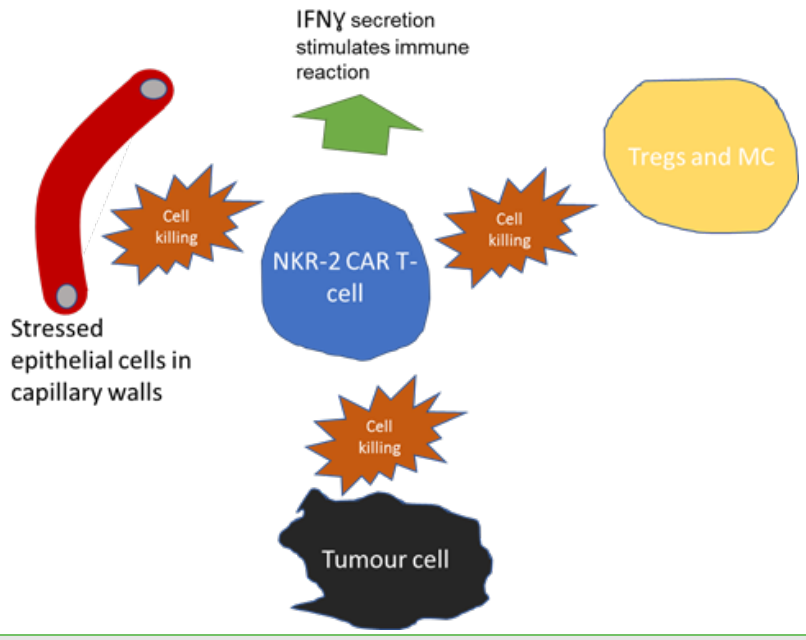
(2006) found the level of soluble MICA antigens in 296 cancer patients at median 161 pg/ml (vs median <30 pg/ml in 62 healthy individuals). This is not massive and is three orders of magnitude below the concentration of 100 ng/ml that Groh *et al* (2002) found was needed for *in vitro* NK cell inhibition by soluble MICA. Sentman has reported (cited in Barber *et al* (2007)) that NKR CAR T-cells are only inhibited by soluble MICA at an amount greater than 1,500 ng/ml, much higher than found in cancer patients.

NKR CAR T-cell efficacy mechanisms

This section is based on preclinical work as clinical evidence is still limited. Exhibit 16 shows key mechanisms of action behind NKR T-cell therapy.

- The NKR T-cells directly attack cancer cells expressing NKG2D ligands; this is a majority of the cells in many solid cancers.
- Tregs which expand to suppress immune activity carry NKG2D-ligands and so are attacked and depleted in the tumour environment (Roy et al (2008)).
- If tumour capillaries have suffered oxidative stress, they can also display NKG2D-ligands and be destroyed. This could be a powerful anti-angiogenic effect and does not require infiltration of the tumour mass by the NKR CAR T-cells.
- Cytokines including IFN and CGM-CSF estimate the host immune response which is important for long-term protection as the NKR CAR T-cells have a limited survival of one to two weeks after infusion. This also gives activity against heterogeneous tumours.

Exhibit 16: NKR T-cells modes of anti-tumour action



Source: Edison Investment Research

There is good evidence in preclinical models for these effects in multiple myeloma and especially ovarian cancer, see Exhibit 17. The doses used were high on a weight basis compared to the proposed THINK maximum dose of $3x10^9$ (3bn). It is not unusual to use high tumour burdens and doses in preclinical models to get fast, clear results.

Exhibit 17: Preclinical efficacy reports

Subject	reference	evidence	dose	comments
Treatment of multiple myeloma	Barber et al 20111	An immune competent mouse strain with murine multiple myeloma	A dose of $5x10^6$ was used; 20 billion cells in a human	Mice given two doses at 5 and 12 days survived and cleared the tumour. Mice were resistant to tumour rechallenge showing a specific memory. Use of lymphodepletion using cyclophosphamide did not improve overall outcomes. CAR T-cells survived for less than 7 days and were detected in organs at levels 100-fold less than the administered dose.
Treatment of ovarian cancer	Barber et al (2008)	An immune competent mouse strain given $2x10^5$ or $5x10^5$ cancer cells		Three injections each one week apart. Mice treated with active NKR CAR T-cells cleared the tumour and gained long-term immunity due to activation of the host immune system.
Anti-angiogenic activity	Zhang and Sentman (2013)2	Mouse tumour vasculature express NKG2D ligands	$2x10^6$	In mice, normal epithelial cells lining capillaries in a tumour are stressed due to reperfusion injury and display Rae1, a mouse equivalent of the human ligand ULBP. Attack by CAR T-cells on the capillaries removes the tumour oxygen supply. IFN^{γ} is needed for activity.
Inhibition of heterogeneous tumours	Spear et al (2013)			Not all tumour cells will express NKG2D ligands so the immune attack needs to spread to cover more antigens and get generalised tumour cell killing.

Source: Edison Investment Research based on cited references

NKR T-cell therapy: Potential off and on target effects

The NKR CAR T-cell approach offers a flexible and potentially safe option to tackle a range of solid cancer types with a wide therapeutic window, Exhibit 18.

Exhibit 18: NKR CAR T-cell technology – off and on target effects

	On target	Off target
On tumour	Preclinical evidence indicates that tumour cells, because they are metabolically and genetically stressed, naturally express high levels of one or more NKG2D ligands.	Preclinical models and some initial patient-specific evidence, suggest that because NKR CAR T-cells do not use preconditioning, antigen spreading and development of natural tumour immunity occurs. This needs to be confirmed in clinical studies. This will be important for long-term efficacy.
	This offers the potential for multiple tumour targeting by NKR CAR T-cells.	
	The availability of up to eight different NKG2D ligand types also reduces the risk of tumour antigen escape.	
	Combination with some chemotherapies (5FU) might potentiate the production of NKG2D ligands. Proteasome inhibitors might also be an effective combination.	
	Checkpoint inhibitor combinations might be evaluated.	
Off tumour	If healthy cells become stressed or damaged, they may express NKG2D ligands so become a target for NKR T-cells. This has not been seen in trials to date but is a theoretical possibility particularly if NKR therapy is combined with chemotherapy or radiotherapy. The SHRINK trial will investigate this aspect.	NKR T-cells also (in mouse models) target tumour vasculature which produces NK-ligands due to oxidative stress. This could give an anti-angiogenic effect if tumour blood vessels are destroyed. This needs to be proven in human clinical trials. Vasculature outside the tumour mass does not appear to express NKG2D ligands.
	Preclinical work discussed below indicates that this is unlikely below a cell dose of 70 billion cells (7x1010)	
	Positively, NKR CAR T-cells show low persistence of up to about 7 days; clinical data still required. This could offer enhanced safety but does require multiple dosing.	

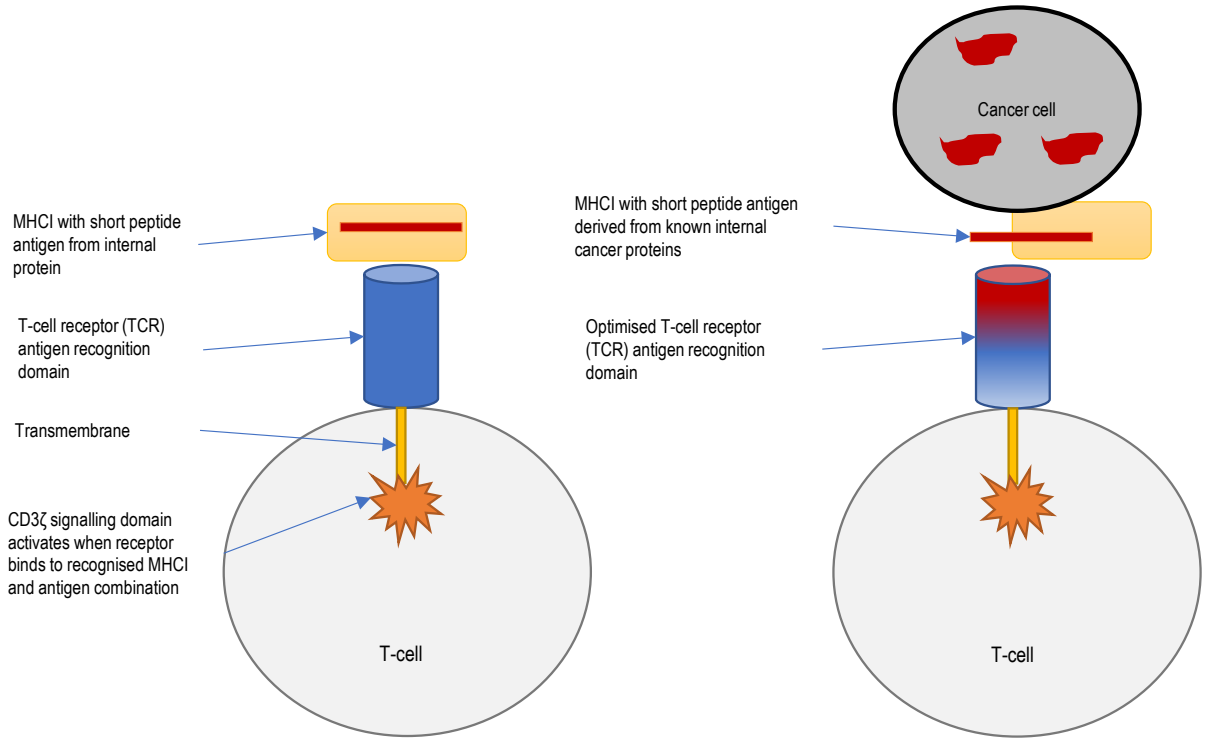
Source: Edison Investment Research

The NKR approach avoids the main limitations of standard CAR-T: lack of selective tumour antigens and inherent toxicity. A potential anti-angiogenic effect and generation of long-term cancer immunity are attractive potential benefits. The current NKR CAR T-cell limitation is that clinical efficacy data is not yet available. The current THINK dose escalation phase and follow on cohort expansions should provide robust dose data with evidence in individual cancer indications.

Section 4: T-cell receptors - examining the entrails

Only TCRs can detect cancer antigens present inside the cell; CAR-T and NKR CAR T-cells never detect these as they only bind larger surface antigens. Being able to see internal antigens opens up a whole class of fetal antigens not seen otherwise on the cell surface and made only by cancer cells in adults. The approach uses the existing T-cell receptor system. The process is shown schematically in Exhibit 19. Exhibit 20 covers terms in TCR therapy.

Exhibit 19: TCR design



Source: Edison Investment Research. Note: There are six CD3 co-stimulatory domains attached to the TCR but only one is shown.

Exhibit 20: MHC, peptides and cancer, background biology

Aspect	Commentary
MHCI (also called Class I HLA)	Displays peptide fragments from proteins from within the cell. Class I molecules that are not recognised as self will trigger a rapid killer T-cell response (by CD8 T-cells). Class I HLA genes HLA-A, -B and -C and their subtypes are expressed by all cells.
Class II HLA or MHCII	Displays variable peptides from fragments of destroyed bacteria, parasites, viruses and foreign cells so not usually relevant to cancer. However, some cancer antigens also elicit an antibody response and this happens via the Class II route.
Peptide fragment	Cells contain tens of thousands of proteins made from strings (polymers) of amino acids. Proteins are constantly synthesised and degraded. As proteins are broken down, short linear peptides of typically 9 amino acids are produced. Some of these are assembled with MHCI and shown on the cell surface. As there are 20 amino acid types, there are 10^{13} ways of assembling a 9 amino acid peptide chain. However, in reality, most of these are never made naturally as modular protein structures are conserved.
T-cell receptor (TCR)	This is made of several interacting proteins. They are hyper-variable through a natural process of gene variation to respond to unpredictable infection threats. T-cells with TCRs that recognise "self" MHC-peptide combinations are destroyed so only T-cells with TCRs that recognise non-self MHC-peptide combinations survive. One TCR clone only recognises the combination of a specific peptide in a specific MHC type. However, although a TCR can recognise a single change in a specific peptide on a specific MHC I type, it might recognise a million other such combinations. The chance of any random match is still one in ten million.
Co-stimulation	A "co-stimulation" is needed to activate the cell. In endogenous T-cells, this is through a second ligand receptor system, often CD80 on the target. Activation of the TCR alone just induces anergy (see below).
Anergy	T-cells have a regulatory mechanism called anergy, where the T-cell recognises an MHC but the co-stimulatory responses are not received so the cell is not activated. This induces tolerance so the T-cell remains alive but quiescent.

Source: Edison Investment Research

Companies select a natural TCR and optimise its genes to improve its binding and selectivity. TCR affinity optimisation needs to be just right – too strong and the T-cells do not activate, too weak and they do not bind. All the complexities of co-stimulation and activation are already in place. We also

know that a $CD8^+$ T-cell attack on any tissue can be powerful, rapid and highly effective: an uncontrolled transplant rejection reaction rapidly destroys a whole organ.

However, there are limitations to TCR therapy paradoxically because TCRs are both highly specific and very promiscuous so detect other antigens.

High specificity…

The immune system uses HLA molecules (human leukocyte antigen or MHCI) to distinguish cells that are healthy and self from infected self or non-self. Choo (2007) presents a good overview. There are two elements: a HLA protein that sits on the cell surface and a presented short peptide lying along a groove in the MHC surface. The peptide is typically nine amino acids long. As there are 20 amino acid types, there are 10^{13} (ten trillion) possible peptide chains. These peptides are generated by proteases as proteins are recycled. Peptides are randomly selected if they fit into a particular MHC. So the antigens presented by a cell to passing T-cells depend on its inherited MHC types and proteins it is making.

The HLA system is very polymorphic with HLA-A2:02 as the most common type (about 47% of people) so that HLA is often targeted for the first product. Each TCR binds to a specific HLA type so each HLA type in the population is a separate development project. As a result, companies will need multiple products to cover the majority of common HLA types.

Although TCR specificity sounds limiting, as many internal cancer antigens are common across cancer types, once a TCR against that antigen in a HLA type is approved, it could be used, in theory, against any cancer showing that antigen and HLA type. The efficacy in different indications may vary but an effective TCR may still have wide off-label use. However, regulators and payors will need to accept treatment of an antigen-HLA combination rather than a traditional indication. The antigen need not be in all the cancer cells since T-cells generate strong inflammatory responses.

…detects many combinations

Although a T-cell receptor can recognise a single change in a specific peptide, each T-cell receptor type might recognise 10^6 (million) possible MHC-peptide combinations, see the commentary by Sewell 2012. This enables the immune system to function with a few million T-cell clones rather than billions. It also means that a TCR could unpredictably recognise other targets. This has toxicity implications. It has happened, Exhibit 21.

Exhibit 21: Early serious clinical side effects in TCR T-cell therapies

Target	Clinical context	Side effects	Reference
MAGE A3 HL:A-A1	This was a TCR study against MAGE A3 – a fetal cancer antigen. Patients had metastatic cancer, mostly melanoma. Of the nine treated, five had remission with two long-term responders.	TCR modified T-cells also reacted against MAGE A12 in brain tissue causing three adverse reactions with two deaths.	Morgan (2013)
MAGE A3	A TCR targeting myeloma showed high specificity in preclinical testing. However, it was tested for off-target binding against different tissue cells grown in culture.	The TCR recognised a totally unrelated protein, titin found inside beating heart muscle. This caused fatal cardiac failure in two patients within five days of administration.	Linette et al (2013)

Source: Edison Investment Research based on cited references

TCR antigens

Most TCRs in development against cancer detect fetal antigens, that is, internal proteins used to enable the embryo to develop and not expressed in normal adult cells. Cancer cells produce fetal proteins as they become malignant. TCR targets in clinical development are in Exhibit 22. *In vivo*, TCRs are produced that bind fragments of mutated proteins so targeting the genetically damaged cell. Unless such mutations are standard and very common, they would not be suitable for commercial development. They can be potentially indirectly targeted by use of checkpoint inhibitors.

Exhibit 22: Current TCR antigens

Antigen	Comments
gp100	Glycoprotein 100. A known marker for melanoma, relatively abundant, with a fragment presented in MHCI (see Bianchi et al (2016)).
NY-ESO-1 I	New York esophageal squamous cell carcinoma-1 is a highly immunogenic cancer peptide which has been well characterised. The protein is normally only found on sperm producing cells in the testes in adults so is also called testis cancer antigen. It has a long history of evaluation for prostate and other cancers like triple negative breast and melanoma (see Krishnadas et al (2013) for a review).
MAGE A10	Melanoma-associated antigen are proteins found in the cell nucleus and uprated in several cancers. As a nuclear protein, it can only be detected by a TCR. Schultz-Thater et al (2011) provide an overview. It is one of 12 in the MAGE cancer testis antigen family.
MAGE-A4	Another MAGE family member but at an earlier stage. A set of trials by Adaptimmune in diverse solid tumours are expected to start during 2017.
MAGE A1	Proposed Medigene study starring perhaps 2017.
AFP	alpha-fetoprotein is being used in a trial hepatocellular cancer. Marker of liver abnormalities.
PRAME	Preferentially-expressed antigen in melanoma, a common cancer antigen. It may alter retinoic acid receptor signalling, a known growth signal (see Epping and Bernards (2006)).
MAGE A3	Was tested, is very immunogenic but showed off target toxicity in brain and heart in two different formats. Is now being used by Kite Pharma in an exploratory study, NCT03139370.

Source: Edison Investment Research and references

A T-cell that recognises a particular MHC-peptide combination becomes activated and kills the cell. In theory, only 5-10 recognised MHCI-peptide complexes trigger a T-cell destruct response. TCR can therefore be very sensitive as well as extremely specific, and very deadly to cancers.

A factor in developing TCR therapies is that the natural TCR affinity for its target is very low; typically TCRs bind to an MHC I-peptide for less than half a second before moving to a new receptor. This is important for the way T-cells become activated: if the engineered, optimised TCR affinity is too strong, the T-cell becomes stuck – and does not respond. Consequently, over engineering the affinity modified TCR construct reduces efficacy but does also reduce the risk of off target binding and so toxicity. By contrast, CARs, being antibody based, stick on firmly, as do NKG2D receptors to their ligands.

Products in development

The commercial field is limited to Bellicum (one exploratory study), Juno (a suspended study) and Adaptimmune in trials. Adaptimmune as a TCR specialist is presented as a small case study in Exhibit 23. Trials are not easy to recruit: Juno wanted 20 patients for its WT1 study and found nine. MediGene has stated that it plans to start clinical studies in haematological cancer in 2017 using natural TCRs without affinity maturation. Bellicum might run a melanoma study. Immunocore bridges the TCR and BiTE areas so is covered in this section. It has a soluble TCR targeting gp100 linked to a CD3 binding arm to bind and activate any passing T-cell to kill the cancer cell. Immunocore's first data in melanoma are due in 2017.

Exhibit 23: Adaptimmune: a TCR specialist

Aspect	Commentary
Antigens	Adaptimmune uses a range of cancer testes antigens particularly MAGE and NY-ESO-1 with a broad programme in a variety of solid tumours. The NY-ESO-1 clinical programme is under option by GSK.
Preconditioning and toxicity	Adaptimmune uses preconditioning with its TCR therapies. The potential for off tumour side effects, both on and off target, remains a concern for the moment but the approach does not seem to show the neurotoxicity and cytokine storm effects seen in CD19 CAR approaches (March 2017 corporate presentation pages 12 and 13).
Overcoming tolerance	SPEAR technology aims to prevent the T-cells becoming inactive due to TGFβ (transforming growth factor beta) produced by the tumour (see Tumour defences, Exhibit 3 above).
Studies and data	There is initial promising data from the Phase I/II Synovial sarcoma and myeloma studies. The sarcoma study might move into registration in 2018 as it is a very rare cancer (1-3 cases per million, perhaps 500-750 cases in the US each year) with a strong unmet need, mostly in young adults if full resection has not been curative. Clinical data is expected in Q417 although the trial is still recruiting. Clinical data is expected from some other projects in 2017.
Limitations	Only half the patients will have the correct HLA-A2 phenotype to be able to utilise the therapy. For a very rare cancer like synovial sarcoma, this makes very tiny numbers but this makes registration more plausible without further studies.

Source: Edison Investment Research

Antigens and cancers targeted are in Exhibit 24.

Exhibit 24: TCR clinical targets and trials

Therapy (NCI score)	Cancer type	Company	Trials	Size	Data due	Comments
Bispecific gp100 TCR-CD3	Melanoma	Immunocore	NCT01211262	84	H1 2017	
	Uveal (eye) melanoma	Immunocore/ AstraZeneca (Medimmune)	NCT02570308	40	Q4 2017	3+3 dose escalation then 20 patient expansion phase.
	Cutaneous melanoma		NCT02535078	180	Q4 2017	combined with Durvalumab and/or Tremelimumab
			NCT03070392	327	Q3 2020	Large follow on to NCT02570308
PRAME	Metastatic uveal melanoma	Bellicum	NA	NA	NA	Planned, currently in AML .BPX-701. incorporates the CaspaCIDe® safety switch
WT-1	Nsclc/ mesothelioma	FHCRC (Juno)	NCT02408016	9	NA	Exploratory dosing, Data Dec 2017. Was 20 patients until August 2017
MAGE A1	Unknown	Medigene	NA	NA	NA	Possible 2017 start
NY-ESO HLA- A2	Synovial sarcoma	Adaptimmune	NCT01343043	65	H2 2017	Target dose 5x10^9.cells. four cohorts testing different or no preconditioning
MAGE A10 HLA A2	Non-small cell lung	Adaptimmune	NCT02592577	32	Q4 2017	Dose escalation to 5x109 with preconditioning
	Bladder, melanoma or head and neck		NCT02989064	22	Q4 2019	Dose escalation with preconditioning
NY-ESO HLA- A2	Multiple myeloma	Adaptimmune/ GSK	NCT01892293	10	Q3 2017	Dose 1x10^{10} cells with mild preconditioning
	Non-small cell lung		NCT02588612	10	Q4 2017	5x10^9 cells with preconditioning
	Advanced myeloma		NCT01352286	26	Q2 2021	Up to 1x10^{10} cells
	Ovarian		NCT01567891	10	Q2 2017	Dose 5x10^9
MAGE A4	Various sold cancers	Adaptimmune	NA	NA	NA	IND open. This construct uses a dummy TGFB receptor to remove soluble TGFB which acts to prevent T-cell activation.
Alpha-fetoprotein (AFP)	Liver	Adaptimmune	NA	NA	NA	IND accepted

Source: Edison Investment Research

TCR: Off and on target effects

TCR products should be very effective due to their selectivity and sensitivity and ability to detect cancer antigens, Exhibit 25. Preconditioning will give TCR T-cell expansion but may make antigen spreading uncertain and this might be more necessary for a longer-term response.

TCR therapy has an unpredictable toxicity risk as TCRs can recognise more than one target, the overall targets could be completely different since only a short peptide from each is bound by the TCR. Exhibit 21 (above) showed two early clinical examples. However, the risks are becoming better understood and pre-screening is intensive. TCR therapy has been in the clinic for some years and data for synovial carcinoma is due in late 2017.

Exhibit 25: TCR technology – off and on target effects

	On target	Off target
On tumour	Could be highly effective and very sensitive due to ability to detect internal cancer-specific antigens. However, less effective if tumour very heterogeneous. Only targets specific HLA type.	Will need a strong host immune response and might need combination with checkpoint products. Use of preconditioning might limit this response.
Off tumour	Could be extremely specific against the designed target peptide antigen is well chosen.	This is a binary issue. If the TCR is carefully optimised and the antigen is very tumour specific, then these products can be highly selective. However, due to TCR promiscuity, it might unexpectedly recognise a totally different peptide fragment.

Source: Edison Investment Research

TCR conclusions – finding niches, seeking roles

TCR therapy development appears complex but the approach targets completely different classes of cancer antigens to CAR-T and NKR CAR approaches. This gives them a differentiated role in therapy. Two standard CAR companies are looking at TCR but neither has done more than limited initial studies. The commercial limitation for some time will be the HLA restriction given the indication-based clinical and payment systems in use. We expect to see much more data over the next two to three years.

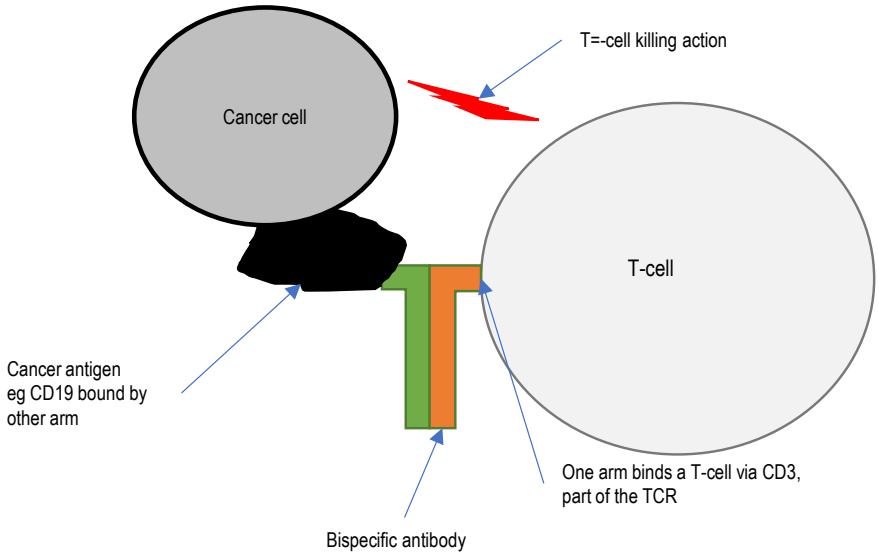
Section 5: Non-cellular therapies involving T-cells

This section looks at two therapies that although not manipulating T-cells directly, rely on native T-cells for their efficacy. The first, BiTEs (Bi-specific T-cell engagers), aims to link cancer cells and any available killer T-cell. The second, checkpoint inhibitors, is a well discussed area with marketed products so this section focuses on the limitations of checkpoint products in solid cancers and the potential for combination therapy.

BiTEing cancer cells: Bi-specific T-cell engagers

Bi-specific antibodies overall is a large development area so this discussion is restricted to BiTEs. These are engineered antibody constructs that link a specific cancer cell antigen to any passing T-cell, Exhibit 26. Typically, one arm binds a cancer cell while the other arm is a CD3 binding arm to capture and activate a T-cell and so triggering it to destroy the cancer cell. The cancer binding arm in a BiTE is usually against a standard cancer antigen but could be a TCR – as with Immunocore's approach (Immunocore is discussed under TCRs Section 4 (above)). CD3 is a co-stimulatory molecule (actually a complex of four proteins) found on the T-cell surface and responsible for the signalling activity of TCRs.

Exhibit 26: BiTE design



Source: Edison Investment Research

The BiTE approach has major advantages for mainstream pharmaceutical companies (like Roche) which have accumulated considerable experience and significant profits from monoclonal antibody therapies. BiTEs are a natural extension of that expertise. Newer entrants are also looking at the area, like Celyad (with an NK-derived BiTE in preclinical) and Glenmark.

BiTEs can be mass produced using well understood mammalian fermentation systems, stored and shipped frozen with a long shelf life and do not require much expertise to administer. As these products use captured tumour infiltrating T-cells for efficacy, there is no costly cell harvesting, modification and culture which vastly simplifies development and eliminates logistical complexity.

The protein product naturally clears or is degraded removing concerns seen with persistent CAR T-cells. In addition, a haematological BiTE has already been approved for leukaemia: Amgen's Blincyto (blinatumomab) that binds CD19. Exhibit 27 has details of the product including efficacy (CR 42% and relapse-free survival, 5.9 months).

Exhibit 27: Blincyto, the approved BiTE

Aspect	Comments
Indication	"Indicated for the treatment of Philadelphia chromosome-negative relapsed or refractory B-cell precursor acute lymphoblastic leukaemia (ALL)."
Efficacy	Complete response rate (including partial haematological recovery) was 41.6% after two courses. Medium relapse-free survival was 5.9 months.
Half-life	Very short half-life of 1-2 hours so is given by continuous iv infusion adding to complexity.
Market size	There are about 6,000 cases of ALL a year in the US with about 1,500 deaths. About 40% of cases are in adults. About 75% are Philadelphia chromosome-negative. This implies a market of about 500 cases a year.
Price and sales	Reputedly $178,000 US list price, with $115m sales reported by Amgen in 2016, equal to about 650 courses at full price. In the UK, 35µg costs £2,017. One 28-day iv course needs up to 28 µg/day so potentially up to $57,000 (28 vials); the dose escalates from 9µg/day on the first course. Two courses separated by a 2-week interval seems normal.
Approval	Approved by FDA in December 2014 under the accelerated system. The TOWER Phase III (2016 Interim data) and other clinical trials are continuing. Some are testing Blincyto with checkpoint inhibitors. In the EU, it was formally authorised in November 2015. Evaluation of cost effectiveness is ongoing.
Toxicities	Side effects include cytokine release syndrome and neurological toxicity. These are very similar to those seen with CAR T-cell therapy.

Source: Edison Investment Research based on Blincyto website

Antigens and BiTEs

Many BiTEs, like Blincyto, are targeted at haematological cancers with easy access to circulating tumour cells. Exhibit 28 shows the current development pattern, all are Phase I trials apart from the Celyad product which is currently a lower priority given the NKR CAR T-cell trials running.

Exhibit 28: BiTE target antigens

Antigen	Cancer type	Company /academic	Trials	Size	Data due	Comments
B7-H6	leukaemia, lymphoma, melanoma and ovarian	Celyad	Preclinical	NA	NA	B7H6 is an NK ligand to the receptor NKp30 (Wu et al (2015).
B7-H3	Wide variety of solid tumours	Macrogenics	NCT02628535	114	Q4 2018	B7-H3 is a T-cell checkpoint inhibitor (Leitner et al (2009)) Castellanos et al (2017).
Glypican 3	Solid tumours including gastric and oesophageal	Chugai	NCT02748837	125	Q4 2019	ERY974. Glypican 3 is known as a liver cancer marker oncofetal protein (Wang et al (2008)).
HER2	HER2 +ve cancers	Glenmark	NCT02829372	30	Q4 2018	Dose ranging, HER2 is particularly associated with breast cancer.
CEA	Metastatic tumours with CEA expression	Roche	NCT02650713	100	Q1 2018	RO6958688, given with the PD-L1 inhibitor Tencentriq (Atezolizumab).
	Metastatic tumours with CEA>50% expression		NCT02324257	120	Q4 2018	Single agent single and multiple ascending dose study.

Source: Edison Investment Research based on cited studies. Note: NCI score for antigen use based on Cheever et al (2009).

The indication for Celyad's preclinical BiTE is not disclosed but the NK ligand selected B7H6 (the natural target of the NK receptor NKp30) has strong preclinical evidence from melanoma and ovarian cancer models. However, an initial haematological dose study might be preferred.

The BiTE from Macrogenics uses B7-H3, a T-cell inhibitor ligand. The BiTE blocks B7-H3 inhibition of T-cells and also recruits T-cells to destroy the cells carrying the ligand. It is being tested in a variety of solid cancer types.

Glypican 3 is usually associated with liver cancer as a fetal oncogenic marker of cell growth so Chugai's targeting of other solid cancers is interesting.

HER2 is a growth factor and known in breast cancer as the target of Herceptin. The study is small.

Finally, Roche has a complex programme of trials with its CEA-targeted BiTE. It is also combining it with its approved PD-L1 checkpoint inhibitor, Tencentriq (Atezolizumab). This strategy might become more common in future to get enhanced efficacy. CEA is a widespread antigen so off tumour, off target toxicities are possible.

BiTEs: Off and on target effects

BiTEs may suffer some of the limitations seen by monoclonal therapies against solid cancer, namely, lack of tumour specificity, poor tumour penetration leading to poor efficacy and potential immunogenicity. Blincyto targets a haematological cancer with accessible cells.

Solid cancer cells are embedded in intracellular matrix. For efficacy, a BiTE needs to penetrate the convoluted capillary system of a solid tumour, diffuse into the matrix (a slow process for large proteins), bind a cancer cell and then wait for a passing CD8+T-cell – which also has to penetrate the matrix of the tumour. Antigen selection for BiTEs against solid cancers is likely to be an issue.

A further issue is likely to be immunogenicity. As BiTEs are non-self, large proteins, they will be processed by dendritic cells. This was seen in the development of monoclonal antibodies derived from mice where a HAMA (Human Anti-Mouse Antibody) response occurred. This means that the expensive new therapy might be neutralised by antibodies and destroyed after prolonged dosing.

Exhibit 29 shows the balance of BiTE on and off target effect. Currently, there is little data on the performance of the earlier clinical products.

Exhibit 29: BiTE technology

	On target	Off target
On tumour	Could be highly effective against isolated tumour cells. Efficacy in solid tumours not known.	Will need a strong host immune response and might need combination with checkpoint products
Off tumour	Lack of specific solid tumour antigens means that these side-effects are likely to occur.	As the cancer cell binding arm can be affinity optimised, this is probably less likely as an issue.

Source: Edison Investment Research

Checkpoint Inhibitors

Checkpoint inhibitors (CPI): Anti-PD-1, anti-PD-L1 and anti CTLA-4 (Exhibit 30) help in immunogenic cancers like melanoma, non-small cell lung cancer and are approved, successful products. These are in effect T-cell stimulants. This is a well-covered area (Hamanishi et al (2016)) with many developments so is only considered briefly to give context to T-cell approaches.

Exhibit 30: Checkpoint inhibitors

Target	Name	Comments
PD-1	Programmed cell death inhibitor 1	PD-1 is a receptor found on T-cells that prevents them attacking if it binds PD-L1. PD-L1 is made by cancer cells so they can escape T-cell attack. Anti-PD-1 therapy bocks the receptor so the T-cell attack recognised tumour cells. However, even if PD-1 is blocked, T-cells still need to recognise the tumour, and may not do so, and there are other T-cell suppression mechanisms. There is research into combining cancer vaccines (to show the T-cells what to attack) with PD-1 therapy.
PD-L1	Programmed cell death ligand 1	PD-L1 is the ligand to PD-1 and can be found at low levels on normal cells. It is often found at high levels on cancer cells and to stop them being destroy by T-cells. If PD-L1 is blocked by an antibody, then the T-cell PD-1 receptor cannot bind and the T-cell destroys the tumour cell - if it is recognised as foreign or damaged. Anti PD-L1 therapies are being developed in combination with other therapies.
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4	This receptor on T-cells down regulates the ability of the immune system to produce new T-cells clones that recognise new antigens, in this case, on cancer cells. The immune system naturally screens and inhibits any new T-cell clone that recognises a "self" antigen. By blocking CTLA-4, T-cell clones that recognise novel cancer antigens are generated and this can produce a sustained anti-cancer response. However, the immune system still has to recognise the cancer. Anti-CTLA-4 therapy also allows T-cells that recognise healthy tissue to be produced giving side effects, particularly gastrointestinal.

Source: Edison Investment Research

CPIs work best when the cancer mutations are random, severe and so more immunogenic, for example, those caused by UV light (melanoma) or external toxins like tobacco smoke (lung, also renal and bladder). PD-1 also work in classic Hodgkin lymphoma (linked possibly to Epstein Barr virus infection) and head and neck (linked to human papilloma virus or to smoking). This strategy requires the immune system to recognise the cancer (anti-CTLA-4 help here) and attack without the T-cells being killed (PD-1 and PD-L1 inhibitors stop this).

An obvious route forward as T-cell therapies develop is to boost the immune response by combination therapy. One TCR trial is already moving down this route. Unless a major safety issue is found, it is likely to become a common development strategy. However, with the cost of checkpoint inhibitors, smaller companies may have to partner with checkpoint inhibitor owners to afford larger studies.

Products are shown in Exhibit 31: The main indication profiles are shown in Exhibit 32. These are being expanded with more clinical trials. However, it is clear that CPIs have lower responses than T-cell therapies promise and a restricted set of indications. It is possible that T-cell therapies could capture the core melanoma market if they show higher efficacy. The major CPI market is seen as nsclc, put by one US analyst at a $10-15bn opportunity.

Exhibit 31: Checkpoint products

Type	Approved products	Comments
PD-1	Keytruda (pembrolizumab, Merck) Opdivo (Nivolumab, BMS)	Anti PD-1 products are found to be more active but this might be because PD-L1 are only just gaining their first approvals and many indications are ion an accelerated basis so data is limited and clinical trial data is still being acquired in trails. Opdiva missed an nsclc first line indication in 2016 in a broader indication of patients with tumour PD-L1 of ≥5% (low levels of the ligand it is meant to block). Opdiva can be combined with Yervoy for some melanoma mutations.
PD-L1	Tecentriq (atezolizumab, Genentech/Roche), Bavencio (avelumab, Pfizer) durvalumab (AstraZeneca) under FDA review	
CTLA-4	Yervoy (ipilimumab, BMS).	Yervoy is the longest studied and can "cure" about 20% of melanoma patients. Yervoy showed 24% 2-year survival vs 14% in advanced metastatic melanoma.

Source: Edison Investment Research

Exhibit 32: Limited checkpoint indications

Indication	Keytruda	Opdiva	Tecentriq	Bavencio	durvalumab	Yervoy
	PD-1	PD-1	PD-L1	PD-L1	PD-L1	CTLA-4
Advanced melanoma	Y	Y				Y
NSCLC 1st line	Y					
NSCLC 2nd line	Y	Y	Y		T	
Head and neck cancer	Y	Y				
cHL	Y	Y				
Renal		Y				
Urothelial (bladder)		Y	Y		R	
Metastatic Merkel Cell[13]				Y		

Source: Edison Investment Research. Notes: nsclc = non-small cell lung cancer, cHL= classic Hodgkin lymphoma, MMC= Metastatic Merkel Cell. Y= approved by FDA, R= under review, T =- late stage trials. Note the EMA approvals may differ or be in progress and there are national pricing discussions in EU states.

Pricing is, inevitably, high so does provide a benchmark for the emerging T-cell therapies.

- Keytruda costs about $150,000 per year in the US ($12,500 per month). This pricing was pushed back in 2016 after a review in England by NICE who sanctioned use only after better clinical outcomes were shown and an (undisclosed) discount accepted.
- Opdiva is $6,000 per 240mg dose given every two weeks so about $144,000 per year at full price. It has had to offer discounts to get into the UK market (a benchmark for tough pharmaco-economic assessments) and even so was deemed uneconomic in April 2017 on a first assessment for head and neck cancer.
- Yervoy is about $28,000 per dose with four doses in a course so about $112,000 full US price.

Combination: a way to boost efficacy and increase indications?

CPI competitors are restricted to a limited number of cancers and would presumably like to extend their available indications. Yervoy is already being combined with PD-L1 products for higher efficacy. This is more necessary as PD-L1 products are trying to target patients with very low PD-L1 ligand frequencies - even PD-L1 negative as benefit is still claimed. However, PD-L1 status needs a diagnostic test and there are no standard methods or benchmarks; diagnostic test levels do not correlate with clinical responses.

In potentially combining CPIs with standard CAR-T, and TCR, the issue of preconditioning becomes crucial. Why boost the immune system after it has been systematically ablated before therapy? An example of a trial with a combination is the Autolus ALEXANDER study in DLBCL, which gives a single dose of dual CAR-T therapy then adds consolidation with pembrolizumab.

A potentially easier future option is to combine CPIs with NKR CAR T-cell therapy. This could work well as no pre-conditioning is used (according to preclinical work) and a generalised immune response is anticipated. The anti-tumour effects and natural immune response generation could be, potentially, stimulated with CPI combinations. BiTEs could also be combined, in theory, as there is an functioning immune system to stimulate but CPIs look more promising, in our view.

[13] A rare type of aggressive skin cancer.

Other technologies: TILs, vaccines and oncolytic virus

Three other approaches will be briefly noted but are, in Edison's assessment, not major threats to commercial development of anti-cancer cell therapies.

Using Tumour infiltrating lymphocytes (TILs)

TILs as a therapeutic concept involves isolating immune cells from a surgically resected tumour mass, culturing them and reinfusing to try to initiate an immune response that breaks tolerance. This was popular in the early period of immune therapy development but with no consistent results. It has logistical issues and is a process not a viable commercial product. A tumour T-cell population will be heterogeneous and can include regulatory T-cells which naturally limit immune responses.

BiTEs need to recruit *in situ* TILs and checkpoint inhibitors seek to activate them. TILs will also be important for endogenous, long-term immune responses in T-cell therapies - although preconditioning will seriously retard these. TILs are important for Celyad's NKR approach.

Cancer vaccines to activate T-cells

Many have sought a simple and effective peptide therapeutic vaccine. yet none have succeeded. The therapeutic prostate cancer vaccine Provenge (Sipuleucel-T) was approved on limited efficacy of 4.1 months additional median survival (Kantoff (2010)). By contrast, prophylactic cancer vaccines against oncoviruses like HPV (cervical cancer) and HBV (hepatitis and liver cancer) are very successful. The issues remain as antigen selection, delivery and overcoming tolerance. Effective T-cell therapies could supersede therapeutic vaccines. There are nonetheless commercial studies that could give approved products. For example, OSE is running a 500 patient Phase III (NCT02654587) of Tedopi (OSE2101) in nsclc. Tedopi is a mixture of ten oncogenic peptides. Data is expected in H118. Note that as a T-cell vaccine, this is specific to HLA-A2 patients.

Note that products that, for example, aim to generate antibody responses against cytokines, receptors or circulating growth factors are not covered.

Oncolytic viruses infecting cancer cells

As cancer cells are fast growing, certain viruses that infect replicating cells can infect them preferentially. The cancer cell might be destroyed as the virus replicates. Infection also makes the cancer cells highly immunogenic. Fukuhara (2016) has reviewed progress.

Only one oncolytic viral product is approved by the FDA for advanced cutaneous and subcutaneous melanoma: Imygic, (talimongene laherparepvec, Amgen). It is given by intralesional injection (so tumours need to be accessible). Imygic showed a 16.3% response rate of which 29.1% were complete responses.

Products in later development include:

- vaccinia virus (pexastimogene devacirepvec) for hepatocellular carcinoma combined with Sorafenib is being evaluated in a 600-patient, Phase III (NCT02562755) by Transgene and its partner SillaJen. Initial data is due in late 2017. Transgene is running combination studies with checkpoint inhibitors.
- Reolysin (pelareorep), a wild-type variant of reovirus, is being progressed by Oncolytics (April; 2017) for metastatic breast cancer based on Phase II data in 74 patients. Reolysin improved overall median ITT survival from 10.4 months to 17.4 months with better subgroup results in p53 mutated cancer in combination with paclitaxel. However, a large Phase III will be needed for approval. Interesting results in other tumour types, like nsclc, have been obtained.

Summary: The solid tumour landscape

The aim of this section has been to outline three cellular and two non-cellular approaches to T-cell therapy. Exhibit 33 summarises these for solid cancers showing advantages and disadvantages of each. The limiting factor is always going to be toxicity and how to balance that against the priority of efficacy.

Exhibit 33: Summary of solid cancer T-cell approaches

Type	Main advantages	Disadvantages	Indications	Antigens	Preconditioning	Side-effects	Persistence and efficacy
CAR-T using antibody targeting	Proven technology in CD19 haematological cancers. Design becoming better understood. Humanised products may give better long-term efficacy.	Complex designs with multiple co-stimulatory domains. humanised scFv needed Side-effect risks may limit to use in specialist centres.	Currently limited exploratory experiments in ovarian, pancreatic and melanoma. Each cancer type needs a new development project	Hard to find cancer specific, cell surface antigens to tackle solid cancer. Trials in China may give additional indications of utility with other antigens like CEA.	Evidence indicates essential requirement to get expansion of infused CAR T-cells and to overcome tolerance. Preconditioning unlikely to affect solid tumours directly.	Risk of targeting healthy tissues, potentially fatal, cytokine release syndrome, neurotoxicity. Possible long-term management issues if cells persist	Long persistence possible (if not yet proved) Additional cytokines may be required for efficacy (IL12). Dual CAR therapy to prevent antigen escape
NKR CAR T-cells	Applicable to multiple types so centres have a possible "universal" cancer treatment. One "CAR" construct needed.	Limited data so far Risk of attack on stressed healthy tissues.	Ovarian; Triple negative breast; Bladder; Pancreatic; and colorectal	Eight possible natural ligands made by stressed cells especially cancer cells and epithelial cells lining tumour blood vessels. Unstressed, healthy cells do not express NKG2D ligands	Preclinical work indicates that the patient's immune system needs to be intact to develop a sustained immune memory. This avoids preconditioning and the complications and toxicities involved.	None seen so far but published data only on low dose range to 30m cells. Preclinical data indicates off target effects only at very high human doses	Low cell persistence to about 7 days. Efficacy to be determined but excellent in preclinical models. Sustained immune responses in preclinical models
TCR T-cell therapy	Only way to detect cancer antigens inside cells. Very sensitive: 5-10 antigen fragments can trigger a kill signal. Very specific: one mutation in a peptide can trigger a kill.	Restricted to patients with specific HLA genes, typically HLA A2 covering about half the population. Other HLA types can be added but are additional projects with diminishing returns.	Initial exploratory trials	Can detect mutated and fetal proteins inside the cell. However, mutation needs to be common. One natural TCR can bind up to a million possible targets	Being used with preconditioning; the most advanced study (Adaptimmune, sarcoma study) is testing different regimen	TCR can be optimised to bind more selectively to avoid off target effects but too strong an affinity removes the T-cell killer response	Could be very powerful. First major trial data in 2017-2018. Cells could be persistent. Development has been prolonged. Multiple products required to cover most patients
BiTEs	Fits standard industry paradigm for mass produced product with repeat doses. Product approved 2009. Avoids complexities and costs of cells.	Relies on captured T-cells killing the tethered cancer cell. As artificial proteins, they are immunogenic which could curtail the length of use.	Many variations depending on construct used and antigen selected.	On the cancer cell, one arm binds an external cell surface antigen or a HLA presented peptide antigen using a TCR. Other arm is an anti-CD3 to capture and activate T-cells	N/A.	Seems to be similar to CAR but limited current data. Off target side effects possible depending on antigen selection	Blinatumomab has a 1-2 hr half-life so needs continuous iv infusion. Monoclonal antibody products can have a 4-06 week duration of action
CPI	Approved products offering improved survival	Good efficacy and survival gains	Restricted indications, mainly nsclc, melanoma and bladder	N/A	No	Yes, but manageable	N/A

Source: Edison Investment Research

The current investment focus on CD19 CAR-T haematological cancers, plus interest in BCMA therapies for MM is logical given their advanced development status. CD19 is still a very new therapy class and there are still many issues not really resolved despite the first approved product. However, they address only a small segment, under 10%, of the overall cancer incidence.

The main investment prize will be in therapies that show enhanced survival in solid tumours; 90% of cases and most unmet medical need, with manufacturing scale and scope. It is not certain, and possibly improbable, that standard CAR-T can move into solid tumours.

The most promising new technology in our view is the natural killer receptor-based CAR T-cell approach pioneered by Celyad. This offers one ubiquitous receptor against multiple ligands expressed in genetically stressed cancer cells. It might be synergistic with chemotherapy, radiation therapy and other products like proteasome inhibitors and CPIs. The use of one CAR-type viral vectors offers some scale economy across multiple tumour types and there is a patented allogeneic approach. The current uncertainties are the need to established clear signs of efficacy in one or more solid cancer types and to define the toxic dose and level of side effects - as yet unseen. So far, no other immune technology for solid cancers has yet emerged that is capable of generating the same type of results as seen in CD19 leukaemia trials.

TCR therapies should be highly effective and access internal and selective set antigens. So far, TCRs have not produced strong efficacy data although much more data will emerge in the next few years. Adaptimune is the leading company in the area. Interestingly, some of the CD19 leaders are using TCR technology to make initial investment in solid tumour T-cell therapy. T-cells are certainly capable of eliminating tumours if they are correctly targeted and activated. However, TCR therapies are very selective and will be restricted to economically feasible HLA (human leukocyte antigen) patient types. They are unlikely to be able to address all potential patients.

BiTEs may be useful, like monoclonal antibodies, in haematological cancers with good tumour access and clear antigens. They have the advantage of manufacturing scale and lower costs but suffer from a lack of clear solid tumour antigens and uncertainties about their efficacy.

Checkpoint inhibitors are approved but on their own have limitations and are breakthroughs because of the paucity of other options in intractable cancers like nsclc. CPIs could however, be essential to boosting the response rates and duration of remission for T-cell therapies.

Given the number of potential patients, prices for T-cell therapy need to become more affordable. This indicates that efficient allogeneic manufacturing will need to be developed and scaled up. Patents on key technologies in allogeneic T-cell therapies are tightly controlled with Celyad holding a core patent.

Core message

Investors now need to look outside the narrow, competitive CD19 area, for the next set of opportunities. Solid cancers therapies will be harder to develop, response rates are unlikely to be as spectacular as CD19 and the key to clinical success might be control and remission. Yet the size of the market will make even partial success worthwhile, in human and investment terms.

Appendix 1: T-cell therapy terminology

The immune system has multiple cell types, complex signalling systems and is carefully regulated. Exhibit 9 gives a relatively non-scientific compilation of the cells and terms involved.

Exhibit 34: The components of the immune system needed for immune therapy

Name	Description
ALL	Acute lymphoid leukaemia – a fast growing cancer of the progenitor cells that make immune B and T cells. The cancer cells spill from the bone marrow into the blood. Patients become anaemic as few red blood cells made. Marked by CD19 antigens on their surfaces
Allogeneic	Cells (including CAR T-cells) taken from one individual, treated and infused into a different person. This is in development for CAR T-cells and is the key to mass produced cheaper therapies. This is the best form of stem cell transplant as it tends to eliminate any residual blood cancer cells in the host.
AML	Acute myeloid leukaemia - a fast growing cancer of the cells that make Natural killer and red blood cells amongst others. The immature cancer cells spill from the bone marrow into the blood. Patients become anaemic. Do not make CD19 so other antigens needed
Antibodies/B-cells/ Plasma cells	Antibodies (Ab) proteins that tightly bind antigens and are created by immature B-cells, another immune system cell type. They are produced by mature B-cells called plasma cells. Ab can have very tight binding to their target so can be made very specific.
Antigen/ligand	Any protein or large molecule recognised by the immune system. Strictly, a ligand is any molecule that binds to a receptor so NKG2D as a receptor and binds ligands. An antigen is a protein bound by an antibody or a peptide-MHC bound by a TCR.
Autologous	Cells extracted from a patient's blood, modified with CAR or TCR, cultured (expanded) and infused back into the same patient.
BiTE (Bi-specific T-cell engager)	A bi-specific antibody where one arm binds a cancer antigen. The other is a CD3 arm the binds and engages a CD8+ T-cell. Can be made more or less antibody like. The cancer antigen arm can be replaced by a TCR in one technology iteration.
Cancer or tumour antigen	An antigen seen mostly, ideally only, on the surface of a cancer cell that can be recognised by an antibody or immune cell. As cancer cells are human, it is hard to find antigens only shown by cancer cells and not by normal cells. However, cancers may make much more of some antigens than healthy tissues. Cancer cells also produce embryonic proteins (also called testis antigens) that are not made by adult cells. These fetal proteins are internal to the cells so can only be seen by TCRs.
Cancer vaccine	A peptide used to generate an immune response against a cancer. Many have been tried but they do not work consistently.
CAR-T therapy	A modified CD8+ T-cell with a Chimeric antigen receptor (CAR) added by insertion of synthetic genes to immune cells isolated from the patient's blood. Expensive customised therapy with high risk of side effects but can be highly potent.
Dual CAR	Gives two different CAR constructs allowing two antigens to be targeted by one T-cell.
CD8+ T-cell	A powerful immune cell that if activated by its TCR binding to a specific peptide antigen shown in MHCI will kill tumour cells. Dangerous, potentially fatal, if out of control. Only needs to see 5-10 peptides to kill.
CD19	Antigen found only on cancerous and healthy B-cells. It enables normal B-cells to develop new antibody types.
Checkpoint inhibitors	Checkpoint proteins are natural protein signals that constrain the immune response. These help tolerance to cancer. Checkpoint inhibitors are antibody therapies that reduce tolerance enabling cancer cells in some patients to attack the tumour cells.
Chimeric antigen receptor (CAR)	An antibody-like artificial front end (usually scFv) outside the cell linked to a TCR signalling module inside the cell. If the front end binds its antigen target, the back end triggers the T-cell to attack. A CAR binds a specific cell surface antigen.
CRS Neurotoxicity	Cytokine release syndrome – uncontrolled release of inflammatory signals by over-excited CAR T-cells. Can be control by an antibody drug that binds IL-6. Can also be fatal. Immune reaction in brain to excessive inflammatory signals, linked to CRS.
Cytokines	Potent chemical protein messenger signals released by activated immune cells to trigger inflammation and stimulate other immune cells to attack.
GvHD	Graft vs Host disease. This is when T-cells infused from another individual react against the host healthy tissues. These T-cells will then rapidly grow and attack organs etc. Can be fatal, Controlled by powerful immune supressing drugs and steroids.
MHCI (HLA)	A complicated and highly variable system for displaying short peptide antigens (fragments of internal cell proteins) on the cell surface. These are recognised by CD8+T-cells using a T-cell receptor (TCR). The advantage is its exquisite sensitivity: a single mutation in a specific MHC I type can be detected. Used in vivo to show that a cell is "self" and healthy, cells that fail the test are immediately killed. Cancer cells show fewer MHC I. MHC I is also called HLA and used for tissue typing in transplantation.
Modified TCR	A genetically engineered TCR recognising a specific peptide antigen on MHCI.. It is implanted into a CD8+ T-cell.
Monoclonal	A single defined (engineered) antibody produced by a cloned cell line in large fermenters for use as therapeutics. Many research uses.
Multiple myeloma	Although this grows in the bone marrow, so is called myeloma and is around the skeleton (multiple), it is a lymphoid cancer of mature plasma cells derived from B-cells. Plasma cells do not show CD19. They do make BCMA (B-cell maturation antigen).
Natural killer cell NK	An immune cell type that detects and kills genetically damaged tissues. These use the natural killer group type 2D system (NKG2D) for detection. There are eight natural ligands. These cells do not attack "self" tissues normally.
NKR CAR	The NKG2D receptor with an added TCR signalling domain added and transplanted into a T-cell. This gives the recognition of an NK cell with the lethal power of a killer T-cell.
NHL/DLBCL	Non-Hodgkin's lymphoma. This is diverse set of white cell cancers similar to ALL. However, these grow in the lymph nodes around the body. DLBCL (diffuse large B-cell Lymphoma) – a subset of 25-30% of NHL cases showing the CD19 antigen.
scFv	Single chain variable fragment of an engineered antibody. Basically, a cut down binding arm of an antibody. Usually initially from mice but then adapted to be more "human" to stop an adverse immune reaction against therapy. Used in CAR-T therapies to target antigens. Can be coupled to others to make, for example, BiTEs.
TCR	T-cell receptor. A multi-part large protein on the surface of T-cells that binds MHC I with associated peptide. There are billions of possible TCRs. One TCR binds one peptide in one type of MHC1 very selectively. They can however, have multiple specificities.
Tolerance	When the patient's immune system recognises but does not attack a tumour despite "recognising" it.
Treg	Regulatory T-cells. Relatively small number of a CD4+ T-cell type that damps down any vigorous immune response. Unchecked immune responses can be fatal – Cytokine released syndrome is an example.
Tumour infiltrating Lymphocytes	Immune cells that recognise the tumour, but tolerate it. Therapeutically, these are extracted from the tumour of a patient, cultured and then reinfused in the usually vain hope that they will then attack the tumour. If activated by, eg Checkpoint inhibitors, can become effective.

Source: Edison Investment Research

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