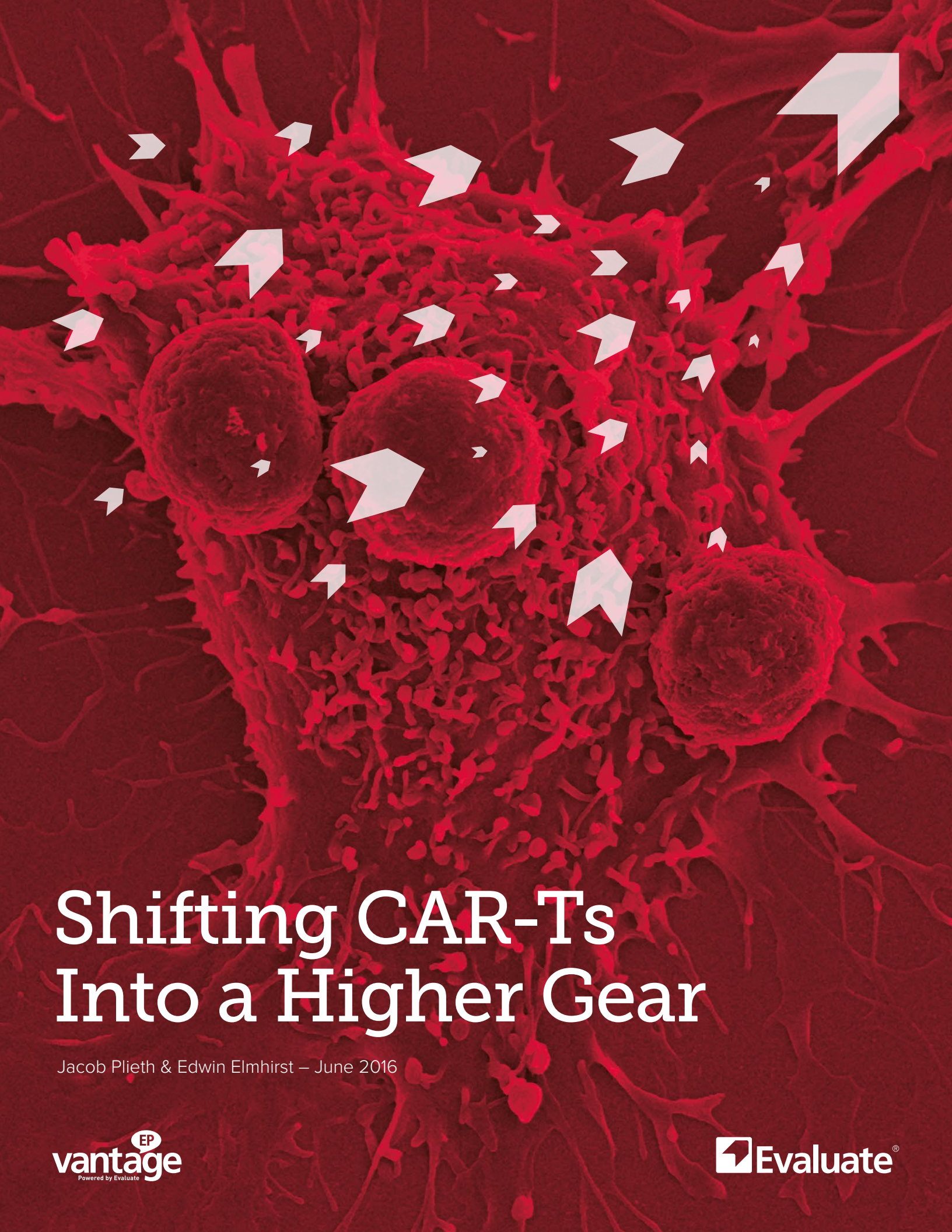

Shifting CAR-Ts Into a Higher Gear

Jacob Plieth & Edwin Elmhirst – June 2016

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Shifting CAR-Ts Into a Higher Gear

After a three-year run-up of mounting excitement over CAR-T therapies comes crunch time. Over the next 12 to 18 months the first couple of leading CAR-T projects could be filed for US approval, and one of them might even secure regulatory go-ahead – if its manufacturer's expectations are realistic.

Some analysts are forecasting blockbuster sales and seeing CAR-T becoming standard therapy in certain relapsed cancers, based on early data that – in certain patients – show long-term activity. These aggressive expectations lie behind two standalone CAR-T companies, Juno and Kite Pharma, boasting billion-dollar market caps, and the bullishness has not abated even as the biotech market turned sour.

Indeed, anyone thinking that the bursting of the bubble might make CAR-T developers take the foot off the gas should look at the 2015 year-end reports from Juno and Kite. Having ended 2015 with $1.2bn and $615m respectively in the bank, the groups this year plan to burn through half a billion dollars between them.

Thus the stage is set for what could be the first commercial US launches of anti-CD19 CAR-T products. After the years of raising cash, securing licensing rights from academia and starting numerous small studies the race is on to generate hard clinical data. Moreover, the sector has not been sitting on its hands as regards outstanding problems, and this report brings up to date the numerous ways in which these are being tackled.

Six CAR-T clinical trials to watch

Source: EP Vantage and company filings

Project	Company	Study	Indication	Timeline	Trial ID
KTE-C19	Kite	Zuma-1	Non-Hodgkin's lymphoma; "pivotal" study	Interim data H2 2016; US filing 2016, US approval 2017	NCT02348216
CTL019	Novartis	Eliana	Paediatric ALL pts	US filing 2017	NCT02435849
JCAR015	Juno	Rocket	Adult ALL pts; "potentially registrational" study	US filing late 2017, US approval 2018	NCT02535364
JCAR017	Juno	–	Non-Hodgkin's lymphoma	US filing 2018	NCT02631044
CTL019	Novartis	Juliet	Diffuse large B-cell lymphoma	–	NCT02445248
KTE-C19	Kite	Zuma-3	Adult ALL pts	–	NCT02614066

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However, it is undeniable that we are in uncharted territory, and it will not be easy for such aggressive development plans to come to fruition; sector bulls cannot ignore the many obstacles that remain.

These include the complexity and expense of manufacturing, the poor durability of many current CAR constructs, the growing ways by which tumour cells can become resistant to therapy, and the ever-present though now somewhat diminished fear of severe toxicities. The expense of CAR-T – estimates are that the cost will be around $500,000 per procedure – looks hard to sustain given that in most cases these treatments have been used as a mere bridge to stem cell transplant.

Commercially, the CD19-focused haematological cancer field has become extremely crowded, necessitating a push into solid tumours – an extremely tough nut to crack owing to their immunosuppressive microenvironment.

But detailed strategies are being drawn up to manage toxicities, and defined-composition products could enable lower doses to be given to improve safety; some groups are working to cut manufacturing costs drastically; ingenious strategies are being developed to overcome relapses, and there is the distant promise of mRNA electroporation and allogeneic CARs.

A huge amount of work has also gone into improving the current generation of CAR constructs, including humanising their antigen-binding domains and boosting stimulatory elements. Novel constructs must also be developed to enable activity in solid tumours, including modular CARs, inhibitory CARs, and products that additionally release cytokines or block immune checkpoints.

This should result in continued corporate finance opportunities as funds are needed to move new projects through pipelines, and collaborative and even M&A activity, as groups work to bring in ever-more revolutionary technologies. Investors still need to proceed cautiously, and it is possible that the coming year will see a major setback, but a sharp revaluation of the space could provide further opportunities.

Report authors | Jacob Plieth and Edwin Elmhirst

Unless stated, all data are sourced to EvaluatePharma and were accurate as of 12 May 2016.

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We've come a long way

Adoptive cell therapy has swept the biotech sector by storm in the past three years, convincing many investors that it heralds a revolution in oncology treatment. However, all of the striking success stories have come from targeting CD19, which is now seen as a low-hanging antigen that offers the key to a limited range of haematological malignancies – a highly competitive therapy space that will now have to be shared between all the leading CAR-T players.

Most companies and investors now agree that the key to longer-term success in this field depends on solving two broad problems: identifying antigens beyond CD19 that can be targeted with CAR-T therapy with strong efficacy, possibly setting up a more proprietary position for their developers than is the case for CD19; and moving beyond haematology into solid tumour indications – a potentially huge market.

This is easier said than done. There are specific reasons why CD19 is a near-uniquely amenable target: it is expressed solely on B cells, whose elimination via CAR-T therapy provides a straightforward route to treating B-cell leukaemias and lymphomas; at the same time loss of the body's B cells is not unduly problematic, as their antibody-generating function can be restored by administering intravenous immunoglobulin (IVIG) to patients.

There are at present very few other targets that offer a similar level of convenience, and it might not come as a surprise that the two that might do are the most advanced non-CD19-directed CAR-T therapies: CD22 in B-cell malignancies and B-cell maturation antigen (BCMA) in multiple myeloma. The former is analogous to CD19, while the latter is an antigen expressed on plasma cells, whose functional loss can also be replaced with IVIG.

The problem of solid tumours is even greater, and so far there is scant evidence of CAR-T being able to overcome the numerous difficulties that exist for these to be targeted efficiently. But, given the potential reward, this has not stopped academic and commercial groups from trying. Assuming that the regulatory hurdles can be overcome the next year or two could see the first launches of CD19-directed CAR-T therapies, after which a huge effort should be directed towards cracking the solid tumour problem.

A report from EP Vantage published last year outlined the key players in the initial explosion of interest in CAR-T therapy, and in the intervening 12 months the list has grown.

Clinical-stage CAR-T projects with commercial licensees (excluding China) Source: EP Vantage and company filings

Company	Academic centre	Project name	Antigen	Co-stim	Transfection*	ScFv	Suicide gene	Added feature(s)
Aurora	Baylor College of Medicine	AU105	Her2	CD28	retrovirus	murine	none	–
Autolus	University College, London	1RG-CART	GD2	CD28	unknown	unknown	suicide gene cassette	–
Bellicum	Baylor College of Medicine	BPX-601	PSCA	none (1st-gen)	retrovirus	murine	none	GoCAR - separate inducible MyD88/ CD40
Bluebird Bio & Celgene	Baylor College of Medicine	bb2121	BCMA	4-1BB	lentivirus	murine	none	–

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Company	Academic centre	Project name	Antigen	Co-stim	Transfection*	ScFv	Suicide gene	Added feature(s)
Bluebird Bio	Uppsala University	3rd-gen CD19 CAR	CD19	CD28 & 4-1BB	retrovirus	unknown	none	–
Bluebird Bio	Baylor College of Medicine	CD30.CAR	CD30	CD28	gamma-retro-virus	murine	none	–
Cellectis, Pfizer & Servier	University College, London	UCART19	CD19	4-1BB	lentivirus	murine	RQR8	TCRα & CD52 knockout; allogeneic
Cellular Therapeutics Ltd	Christie Hospital NHS Foundation Trust	aCD19z	CD19	none (1st-gen)	retrovirus	murine	none	–
Cellular Therapeutics Ltd	Cancer Research UK	anti-CEA MFEz	CEA	none (1st-gen)	unknown	murine	none	–
Juno	Fred Hutchinson & NCI	JCAR014	CD19	4-1BB	lentivirus	murine	EGFRt	–
Juno	Memorial Sloan Kettering	JCAR015	CD19	CD28	gamma-retrovirus	murine	none	–
Juno	Seattle Children's Hospital	JCAR017	CD19	4-1BB	lentivirus	murine	EGFRt	defined-composition product
Juno, via Opus Bio	NCI	JCAR018	CD22	4-1BB	lentivirus	human	none	–
Juno	Seattle Children's Hospital	JCAR023	L1CAM (=CD171)	4-1BB	lentivirus	murine	EGFRt	–
Juno	Memorial Sloan Kettering	JCAR020	MUC16	CD28	gamma-retrovirus	fully human	EGFRt	IL12-secreting "armored CAR"
Juno	Fred Hutchinson Cancer Center	JCAR024	ROR1	4-1BB	retrovirus	rabbit	EGFRt	might be CD4+:CD8+ defined cell product
Kite Pharma	Zelig Eshhar (Cabaret Biotech)	KTE-C19	CD19	CD28	gamma-retrovirus	murine	none	–
Kite Pharma	NCI	Anti-EGFRvIII CAR	EGFRvIII	CD28	gamma-retrovirus	murine	none	–
Leucid Bio	King's College London	LEU-001 (T1E28z)	ErbB dimers	CD28	gamma-retrovirus	murine	none	IL4 receptor to aid expansion
Mustang (Fortress Bio)	City of Hope Medical Center	MB-102	CD123	CD28	lentivirus	murine	EGFRt	–
Mustang (Fortress Bio)	City of Hope Medical Center	MB-101	IL13Rα2	4-1BB	lentivirus	murine	truncated CD19	–
Novartis	University of Pennsylvania	CART-BCMA	BCMA	4-1BB	lentivirus	murine	none	–
Novartis	University of Pennsylvania	CTL019	CD19	4-1BB	lentivirus	murine	none	–
Novartis	University of Pennsylvania	CTL119	CD19	4-1BB	lentivirus	humanised	none	–
Novartis	University of Pennsylvania	CART22 cells	CD22	4-1BB	lentivirus	murine	none	–
Novartis	University of Pennsylvania	CART-EGFRvIII	EGFRvIII	4-1BB	lentivirus	murine	none	–
Novartis	University of Pennsylvania	CART-meso	meso-thelin	4-1BB	lentivirus	murine	none	–
Ziopharm, Intrexon & Merck KGaA	MD Anderson Cancer Center	CD19 CAR	CD19	CD28	Sleeping Beauty	murine	none	includes donor-derived matched "allo"

Note: *where retrovirus is stated this is likely a gamma-retrovirus, but since lentiviruses are a subtype of retroviruses it is possible that a lentivirus is being used.

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Lots of deals, but where is big pharma?

There has been significant deal activity in CAR-T, though given the potential it can arguably still be seen as relatively sparse. By and large big pharma and biotech have remained cautious of buying into the space – deterred presumably by the entry price more than the scientific rationale.

It is certain that all big pharma companies have looked at the space. But Daniel O'Day, pharma chief of the world's most important oncology player, Roche, said last November that while the technology was extremely promising the entry prices were too high. Under the stewardship of Chris Viehbacher Sanofi is also understood to have come to the same conclusion. And more recently Gilead's newly appointed chief executive, John Milligan, said the concept of autologous T-cell therapies as commercial products made him "nervous".

Nevertheless, deals have got done. One big pharma player fully invested in CAR-T is Novartis, though its alliance with the University of Pennsylvania dates back to 2012, and being such an early entrant the group's financial outlay has been relatively limited. Pfizer, too, has a hand in the CAR-T space via two deals covering Cellectis's allogeneic projects; the first was an alliance covering up to 15 antigen targets, while the second, routed via Servier after that company surprisingly exercised an early opt-in last November, covers UCART19, which until then had been Cellectis's lead CAR-T asset. The first deal was worth $80m up front; while the signing fee between Pfizer and Servier has never been disclosed, Servier paid Cellectis $38.2m to exercise the opt-in.

By far the biggest bet by a large biopharma company has been made by Celgene, which in 2015 struck a monster deal with Juno worth $150m cash up front plus an $846m equity stake priced at a significant premium. The tie-up, which allows Celgene to opt into virtually Juno's entire pipeline over the next 10 years, is somewhat short on detail, but it represented a watershed moment in CAR-T development: it could either turn out to be a vastly overpriced folly along the lines of Bristol-Myers Squibb's $1bn investment in Imclone in 2001, or a stroke of genius like Roche's ground-breaking Genentech deal in the 1990s.

Clearly, Celgene recognised the game-changing potential of CAR-T therapy and paid whatever it took to get its hands on the most advanced player available (Celgene goes for broke, June 30, 2015). Celgene had had a broad CAR-T tie-up with Bluebird Bio, based around technology derived from Baylor College, but this was scaled back after the Juno deal, and is now effectively limited to the anti-BCMA asset bb2121.

Earlier last year Merck KGaA accessed MD Anderson Cancer Center's Sleeping Beauty-based CAR-T technology via a $115m payment to its licensees, Intrexon and Ziopharm Oncology. The up-front fee equalled that paid by the two licensees to MD Anderson just two months earlier, though as a whole all of these parties are arriving to the CAR-T party rather late in the day (German Merck widens oncology presence with CAR-T deal, March 31, 2015).

Another big biotech that has backed CAR-T is Amgen, though its bet is much more cautious than Celgene's. Amgen's collaboration is with Kite Pharma, but is effectively an early-stage discovery alliance covering novel CAR constructs. Amgen will provide new haematological and solid tumour targets, with Kite leading pre-IND work including cell manufacturing. Amgen paid Kite a $60m signing fee (Amgen takes the CAR for a spin, January 6, 2015).

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Apart from payments by corporates to get their hands on academic work in CAR-T there have also been numerous small but potentially important technology deals struck, including the following:

Key CAR-T technology deals

Source: EP Vantage

Company	Partner	Deal notes
Juno	Eureka Therapeutics	Covers a fully human ScFv binding domain.
Juno	Stage Cell Therapeutics	Acquisition to gain access to a novel manufacturing process.
Juno	Editas Medicine	Covers Crispr/Cas9 genome-editing technology.
Kite	Alpine Immune Sciences	Access to Alpine's transmembrane immunomodulatory protein technology.
Johnson & Johnson	Transposagen	Rights to Piggybac footprint-free genome-editing technology to develop allogeneic CAR-T therapies.
Johnson & Johnson	Poseida Therapeutics (a Transposagen spin-out)	Focuses on J&J's Centyrin technology to develop CAR-T binding domains made up of alternative scaffold molecules rather than antibody-derived ScFv regions.
Baxalta	Precision Biosciences	Use of use Arcus nuclease genome-editing technology to develop allogeneic CAR-T therapies.
Novartis	Intellia Therapeutics and Caribou Biosciences	Use of Crispr/Cas9 genome-editing to develop allogeneic CAR-T therapies.

The obvious question from a corporate finance point of view, therefore, is what further deals might get done. Small, early-stage discovery-type tie-ups are a near certainty, especially given the private start-ups constantly springing up with novel technologies, though deal bankers will likely be looking for something bigger. In the current market, however, it is hard to see a big pharma player or a large biotech making the sort of endorsement that Celgene made last year unless new stunning data are revealed, or if for whatever reasons (eg, a setback) valuations take a sharp knock. Given the bullish expectations, and the promises made by the leading trio in the face of the continuing unknowns to bring CAR-T therapies to market sooner rather than later, this can by no means be ruled out.

It is also worth considering whether any deals or even consolidation could take place among the current CAR-T players. One hint was recently dropped by Juno's chief scientific officer, Hy Levitsky, who at the AACR meeting in April expressed his group's desire to own a CAR-T suicide switch that is neater and less dangerous to use than Juno's current EGFRt technology, which no doctor has dared use in the clinic for fear of causing an inflammatory response.

Could Juno therefore buy Bellicum, which has developed such a suicide technology? Mr Levitsky accepts the severe limitations of Juno's EGFRt switch, and at the time declared an interest in licensing or perhaps even buying Bellicum's. "It all comes down to how the two parties view the value of the asset, but if that can be broached then I think [a deal] would make a lot of sense," he said.

Bellicum has already floated the idea of licensing its CAR-T suicide switch to other industry players, and while it might appear to make little sense to license it to an arch rival like Juno, Bellicum has lost over half its value in the past year, so its bargaining power is much reduced. Moreover, Juno could get a first-hand look at Bellicum's suicide technology from a trial its partner, Memorial Sloan Kettering Cancer Center, is running that paradoxically incorporates it, though Mr Levitsky stresses that this is under a purely academic arrangement.

But he cautions that there are some issues with using rimiducid, on which Bellicum's technology is based, adding: "There is a lot of interest in using other kinds of drugs for this. We're working on our own drug-inducible technology." (AACR interview – Juno's search for bells and whistles, April 22, 2016).

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It is also worth noting that Clinicaltrials.gov reveals numerous CAR-T projects that are apparently still in the sole hands of academia.

Clinical CAR-T constructs with sole involvement from academia (excluding China)

Source: EP Vantage, scientific literature

Academic centre	Project name	Antigen	Co-stim	Transfection*	ScFv	Suicide gene	Added feature(s)
Baylor College & NCI	VZV-specific GD2 CAR	GD2	CD28 & Ox40	retrovirus	murine	iC9	–
Baylor College of Medicine	Kappa-CD28 T cells	unknown	CD28	retrovirus	murine	none	–
Baylor College of Medicine	Virus-specific CD19.CAR	CD19	CD28	gamma-retrovirus	murine	none	–
Baylor College of Medicine	EBV-specific CAR.CD30	CD30	CD28	gamma-retrovirus	murine	none	–
Baylor College of Medicine	GINAKIT Cells	GD2	CD28 & Ox40	retrovirus	murine	iC9	uses natural killer T cells
Baylor College of Medicine	EBV-specific GD2.CAR	GD2	none (1st-gen)	retrovirus	murine	none	–
Baylor College of Medicine	HER2.CAR VSTs	Her2	CD28	retrovirus	murine	none	EBV T cells
Children's Mercy Hospital Kansas City	EBV-specific GD2.CAR	GD2	unknown	retrovirus	murine	none	–
City of Hope Medical Center & NCI	CD19CAR	CD19	CD28	lentivirus	murine	EGFRt	TCM-enriched CD8+ T cells
City of Hope Medical Center & NCI	anti-IL13 zetakine	IL13Rα2	none (1st-gen)	unknown	murine	HyTK	CD8+ T cells
Duke University Medical Center	EGFRvIII CAR	EGFRvIII	CD28 & 4-1BB	retrovirus	murine	none	–
Fred Hutchinson & NCI	CMV or EBV-specicifc CD19 CAR	CD19	4-1BB	lentivirus	murine	EGFRt	donor-derived "allo"
Fred Hutchinson & NCI	CD20-specific T cells	CD20	CD28 & 4-1BB	mRNA electroporated	murine	none	–
Fred Hutchinson & NCI	CE7R	L1CAM (=CD171)	none (1st-gen)	plasmid	murine	HyTK	CD8+ T cells
MD Anderson Cancer Center	ROR1R-CAR	ROR1	CD28 or 4-1BB	Sleeping Beauty	murine	none	–
Memorial Sloan Kettering	19-28z+	CD19	CD28	mRNA electroporated	murine	none	–
Memorial Sloan Kettering	iCasp9M28z	meso-thelin	CD28	gamma-retrovirus	murine	iC9	–
Memorial Sloan Kettering	autologous T cells	PSMA	CD28	retrovirus	murine	HSV-TK	–
NCI	BCMA CAR	BCMA	CD28	gamma-retrovirus	murine	none	–
NCI	CD19 CAR	CD19	CD28	gamma-retrovirus	murine	none	donor-derived "allo"
NCI	huCD19 CAR**	CD19	CD28	gamma-retrovirus	fully human	none	–
NCI	allogeneic lymphocytes	FRα	none (1st-gen)	gamma-retrovirus	murine	none	–
NCI	GD2 CAR	GD2	CD28 & Ox40	retrovirus	murine	iC9	–

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Academic centre	Project name	Antigen	Co-stim	Transfection*	ScFv	Suicide gene	Added feature(s)
NCI	Her-2 CAR	Her2	CD28 & 4-1BB	gamma-retrovirus	murine	none	–
NCI	mesothelin CAR	meso-thelin	CD28	gamma-retrovirus	murine	none	–
NCI	VEGFR2 CAR	VEGFR2	CD28 & 4-1BB	retrovirus	murine	none	–
Peter MacCallum Cancer Centre, Australia	Anti-LeY CAR	Lewis Y	CD28	retrovirus	murine	none	–
Roger Williams Medical Center	anti-CEA CAR	CEA	CD28	gamma-retrovirus	murine	none	combination with Sir-Spheres
Roger Williams Medical Center	anti-PSMA CAR	PSMA	CD28	gamma-retrovirus	murine	none	–
University College, London	CD19 CAR	CD19	CD28	retrovirus	murine	none	–
University of Cologne	HRS3scFv derived CAR	CD30	unknown	unknown	unknown	unknown	–
University of North Carolina	ATLCAR.CD30	CD30	CD28	retrovirus	murine	none	–
University of Pennsylvania	RNA CD123	CD123	4-1BB	mRNA electroporated	murine	none	–
University of Pennsylvania	RNA CART19	CD19	4-1BB	mRNA electroporated	murine	none	–
University of Pennsylvania	RNA cMet CAR	c-Met	4-1BB	mRNA electroporated	murine	none	–
University of Pennsylvania	RNA Meso-CIR T	meso-thelin	4-1BB	mRNA electroporated	murine	none	–
University of Zurich	FAP-specific T Cells	FAP	CD28	retrovirus	murine	none	CD8+ T cells

Note: *where retrovirus is stated this is likely a gamma-retrovirus, but since lentiviruses are a subtype of retroviruses it is possible that a lentivirus is being used; **CRADA with Kite Pharma.

First to market

Whatever deals still have to be done clinical data are needed, and Kite is most aggressive, stressing that in the second half of 2016 KTE-C19 should yield interim results from its pivotal Zuma-1 lymphoma trial. Juno's JCAR015 has a smaller first indication – adult acute lymphoblastic leukaemia (ALL) – with Juno calling its Rocket study a "potential US registrational trial" that could put the company in a position to file in late 2017.

The distinctly more reticent Novartis, meanwhile, is pursuing another small indication, childhood ALL, as the first indication for CTL019, though its own timeline has slipped – it had initially been targeting a 2016 filing. To date it is Novartis that has generated by far the most data.

It is worth considering, however, how much of an advantage it will be to come first to a market that has never before been tested, and in which the necessary standard of clinical robustness and ability to withstand premium pricing can only be guessed at present. "There are pricing advantages to coming first [to market], but there are some advantages to coming second if you've got a particular benefit in a discrete group of patients," Juno's chief executive, Hans Bishop, has stated (Juno and Kite fight it out to be first to market, March 1, 2016).

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CAR-T design

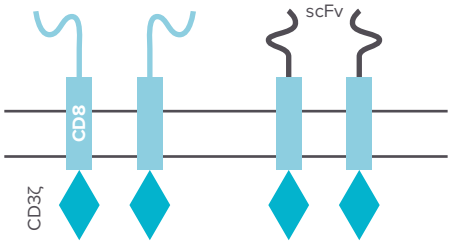
The vast majority of CAR-T projects at present in clinical studies incorporate a so-called second-generation design, following on from the first-generation constructs of the 1990s. These early constructs, sometimes called “T bodies”, had an antigen-binding region derived from an antibody, a transmembrane anchor and a single intracellular stimulatory domain; they were not particularly efficacious.

Second-generation CARs add a second stimulatory region, called a co-stimulatory domain, and this has proved to offer a significant improvement in terms of T cell persistence and ultimately efficacy. The most commonly used co-stimulatory domains are CD28 and 4-1BB (CD137), but also include ICOS, Ox40 and other possibilities.

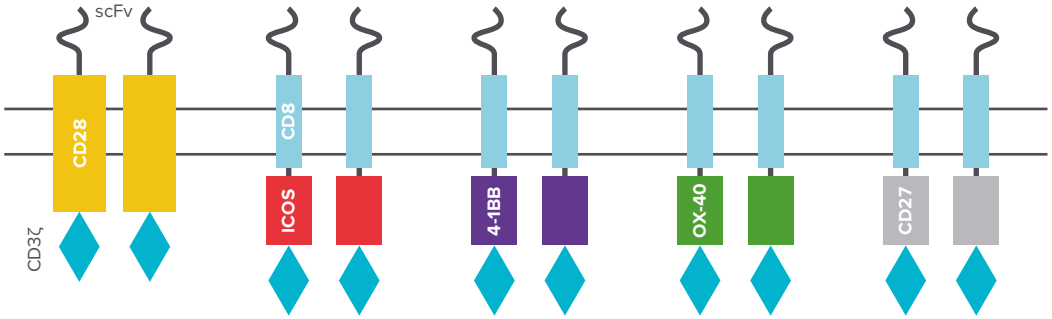
Third-generation constructs incorporate more than one co-stimulatory region, while the fourth generation might be more complex still, though this last type of construct has yet to enter the clinic.

Diagrammatic representation of three generations of CAR constructs

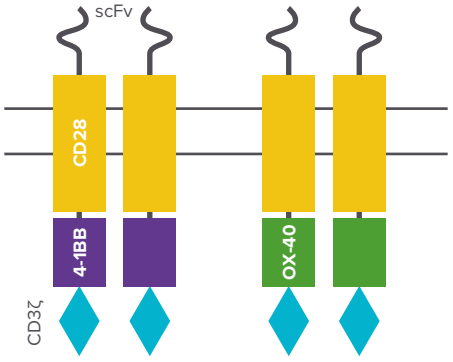
First-generation CAR



Second-generation CAR



Third-generation CAR



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ALL and CD19 – the "easy targets"

By far the greatest amount of work has been done on targeting CD19, a protein expressed on B cells and thus being an especially apt antigen target in haematological cancers involving this cell type, ie, ALL, chronic lymphocytic leukaemia (CLL) and B-cell lymphomas. Within this the most impressive response data generated so far have concerned refractory ALL.

Selected CAR-T clinical data in acute lymphoblastic leukaemia (ALL)

Source: EP Vantage and company filings

Project	Sponsor	Indication	Patients	Initial CR* rate	Relapses?	Trial ID	Presented at
CTL019	Novartis	Childhood r/r ALL	59	93%	ORR drops to 31% at 1 year	NCT02228096	ASH 2015
JCAR015	Juno	Adult r/r ALL	45	82%	18 pts relapsed during follow-up	NCT01044069	MSKCC 2016
CD19 CAR	NCI**	ALL	45	100%	ORR drops to 60% at 1 year	NCT01593696	MSKCC 2016
JCAR017	Juno	r/r ALL	37	92%	6 CD19-ve, 8 CD19+ve relapses	NCT02028455	MSKCC 2016
UCART19	UCL/GOSH***	CD19+ve ALL	2	100%	both still in remission after <1 yr	2 case reports	ASH 2015 & ASGCT 2016

Note: *CR=complete remission; **has CRADA with Kite; ***used Cellectis CAR-T project.

It is obvious, however, that despite the highly impressive remission rates obtained initially in what are difficult-to-treat, refractory patients, subsequent relapses are common, as is toxicity (ASH – CAR-T struggles to travel beyond leukaemia, December 8, 2015).

Nevertheless, this has not stopped active research into CD19-positive haematological diseases, with Clinicaltrials.gov revealing 56 Western studies (including completed and terminated trials) seeking to recruit over 3,000 patients.

Active anti-CD19 CAR-T studies (excluding China)

Source: EP Vantage, Clinicaltrials.gov

Project name	Company	Academic centre	Indication(s)	Enrolment	Trial ID	Trial status
3rd-gen CD19 CAR	Bluebird Bio	Uppsala University	leukaemia and lymphoma	15	NCT02132624	recruiting
UCART19	Cellectis, Pfizer & Servier	University College, London	ALL & CLL	12	NCT02746952	not yet recruiting
UCART19	Cellectis, Pfizer & Servier	University College, London	lymphoid malignancies	200	NCT02735083	not yet recruiting
aCD19z	Cellular Therapeutics Ltd	Christie Hospital NHS Foundation Trust	B-cell leukaemia and lymphoma	24	NCT01493453	recruiting
JCAR014	Juno	Fred Hutchinson & NCI	CLL, ALL & non-Hodgkin's lymphoma	145	NCT01865617	recruiting
JCAR015	Juno	Memorial Sloan Kettering	non-Hodgkin's lymphoma	18	NCT01840566	recruiting
JCAR015	Juno	Memorial Sloan Kettering	ALL	24	NCT01860937	recruiting
JCAR017	Juno	Seattle Children's Hospital	childhood ALL	18	NCT01683279	active, not recruiting
JCAR017	Juno	Seattle Children's Hospital	leukaemia	80	NCT02028455	recruiting

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Project name	Company	Academic centre	Indication(s)	Enrolment	Trial ID	Trial status
JCAR017	Juno	–	non-Hodgkin's lymphoma	70	NCT02631044	recruiting
JCAR015	Juno	–	adult ALL	90	NCT02535364	recruiting
JCAR014 + durvalumab	Juno & AstraZeneca	Fred Hutchinson Cancer Center	non-Hodgkin's lymphoma	42	NCT02706405	not yet recruiting
KTE-C19	Kite Pharma	–	non-Hodgkin's lymphoma	124	NCT02348216	recruiting
KTE-C19	Kite Pharma	–	adult ALL	75	NCT02614066	recruiting
KTE-C19	Kite Pharma	–	paediatric ALL	75	NCT02625480	recruiting
KTE-C19	Kite Pharma	–	mantle cell lymphoma	70	NCT02601313	recruiting
CTL019	Novartis	University of Pennsylvania	CLL	59	NCT01747486	active, not recruiting
CTL019	Novartis	University of Pennsylvania	CLL	15	NCT02640209	recruiting
CTL019	Novartis	University of Pennsylvania	DLBCL	100	NCT02445248	recruiting
CTL019	Novartis	University of Pennsylvania	CD19+ve lymphomas	51	NCT02030834	recruiting
CTL019	Novartis	University of Pennsylvania	ALL	78	NCT02435849	recruiting
CTL019	Novartis	University of Pennsylvania	adult ALL	67	NCT02167360	not yet recruiting
CTL019 & CTL119	Novartis	University of Pennsylvania	paediatric ALL	67	NCT02228096	recruiting
CTL019	Novartis	University of Pennsylvania	ALL	24	NCT02030847	recruiting
CTL019	Novartis	University of Pennsylvania	leukaemia and lymphoma	20	NCT01626495	active, not recruiting
CTL019	Novartis	University of Pennsylvania	various	500	NCT02445222	recruiting
CTL019	Novartis	University of Pennsylvania	multiple myeloma	13	NCT02135406	active, not recruiting
CD19 CAR	Ziopharm, Intrexon & Merck KGaA	MD Anderson Cancer Center	lymphoid malignancies	30	NCT02529813	recruiting
CD19-specific T cell	Ziopharm, Intrexon & Merck KGaA	MD Anderson Cancer Center	CLL	30	NCT01653717	active, not recruiting
CD19.CAR	–	Baylor College of Medicine	CLL, ALL & non-Hodgkin's lymphoma	40	NCT02050347	recruiting
CD19.CAR	–	Baylor College of Medicine	CLL, ALL & non-Hodgkin's lymphoma	14	NCT01853631	recruiting
CD19.CAR	–	Baylor College of Medicine	CLL, ALL & non-Hodgkin's lymphoma	14	NCT00586391	active, not recruiting
Virus-specific CD19.CAR	–	Baylor College of Medicine	various	68	NCT00840853	active, not recruiting
CD19.CAR vs EBV-specific CD19.CAR	–	Baylor College of Medicine	CLL and lymphoma	3	NCT00709033	active, not recruiting
CD19CAR	–	City of Hope Medical Center & NCI	non-Hodgkin's lymphoma	57	NCT01318317	active, not recruiting
CD19CAR	–	City of Hope Medical Center & NCI	non-Hodgkin's lymphoma	30	NCT01815749	active, not recruiting
CD19CAR	–	City of Hope Medical Center & NCI	non-Hodgkin's lymphoma	27	NCT02051257	recruiting
CD19CAR	–	City of Hope Medical Center & NCI	ALL	48	NCT02146924	recruiting

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Project name	Company	Academic centre	Indication(s)	Enrolment	Trial ID	Trial status
CD19CAR	–	City of Hope Medical Center & NCI	lymphoma and CLL	48	NCT02153580	active, not recruiting
19-28z+	–	Memorial Sloan Kettering	ALL	60	NCT01044069	recruiting
autologous lymphocytes	–	Memorial Sloan Kettering & NCI	CLL and lymphoma	30	NCT00466531	recruiting
CD19 CAR	–	NCI	B-cell malignancies, post-transplant "allo"	42	NCT01087294	recruiting
hCD19 CAR	–	NCI	B-cell malignancies	64	NCT02659943	recruiting
CD19 CAR	–	NCI	lymphoma	43	NCT00924326	active, not recruiting
CD19 CAR	–	NCI	paediatric ALL	90	NCT01593696	recruiting
CD19 CAR	–	University College, London	DLBCL	12	NCT02431988	not yet recruiting
CD19 CAR	–	University College, London	haematological malignancies	18	NCT02443831	not yet recruiting
RNA CART19	–	University of Pennsylvania	Hodgkin's lymphoma	16	NCT02277522	recruiting
RNA CART19	–	University of Pennsylvania	Hodgkin's lymphoma	10	NCT02624258	recruiting

Lymphomas – a little tougher to crack

Lymphomas are a much more interesting indication than ALL on account of the larger numbers of patients involved, and some analysts expect them to follow relapsed/refractory ALL as the next target where in some settings CAR-T will become a standard therapy. But they are problematic, needing bigger trials, for instance. While remission rates of 90% are common in ALL, in lymphomas they are closer to 50%, and further treatment optimisation is needed to improve efficacy.

Lymphomas are also harder to treat with CAR-T, possibly owing to their nodal nature and an immune-suppressive tumour microenvironment that renders T cells less active. The theme of low remission rates relative to ALL is obvious.

Selected CAR-T clinical data in non-Hodgkin's lymphoma (NHL)

Source: EP Vantage and company filings

Project	Sponsor	Indication	Patients	Responses*	Trial ID	Presented at
CTL019	Novartis	r/r CD19+ lymphomas	30	14 CRs, 2 PRs	NCT02030834	ASH 2015
JCAR017**	Juno	B-cell NHL cohort	30	10 CRs, 9 PRs	NCT01865617	MSKCC & AACR 2016
CD30.CAR	Baylor College***	CD30+ lymphomas	9	2 CRs, 1 PR	NCT01316146	ASH 2015
KTE-C19	Kite	r/r NHL	7	4 CRs, 1 PR	NCT02348216****	AACR 2016

Note: *PR=partial response; **defined-composition product; ***former Celgene involvement; ****Zuma-1 study.

Other haematological cancers

Beyond CD19 increasing numbers of antigens are being targeted with CAR-T therapies, though because of the much earlier nature of these studies clinical data are virtually non-existent. Two other haematological malignancies that could benefit from CAR-T therapy are relapsed multiple myeloma and acute myelogenous leukaemia (AML).

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In multiple myeloma a promising target is BCMA, a member of the TNF receptor superfamily expressed by plasma cells and some mature B cells. Three US studies are ongoing, two of these with the involvement of the NCI's Dr James Kochenderfer.

In the NCI's own trial he has treated 12 multiple myeloma patients so far, and results have been somewhat mixed: three partial responses, and one stringent complete response, with the CAR-T therapy able completely to eradicate malignant plasma cells in this complete response patient. There seems to be correlation with dosing, with two of the responses coming from three patients given the highest dose of cells, though safety needs to be watched: patients on the highest dose of CAR-T cells experienced cytokine release syndrome (CRS), as well as muscle pain, and heart and kidney problems. The obvious question is whether a therapeutic window exists with this type of therapy, though Dr Kochenderfer said CRS was manageable and reversible, with two patients being given the anti-IL6 MAb Actemra to treat it.

However, at the Memorial Sloan Kettering CAR-T seminar in March Dr Kochenderfer reported that the stringent complete response patient relapsed after 17 weeks. It will be crucial to estimating the potential of BCMA to learn more about this relapse – was it due to waning cell persistence or loss of the antigen, for instance? At present Dr Kochenderfer says he is still investigating the precise nature of the relapse.

He is separately primary investigator of a recently initiated trial of Bluebird's anti-BCMA CAR-T, bb2121. There are differences between the two constructs, and while the NCI uses gamma-retroviral transfection Bluebird employs lentiviral technology (ASH – US academic studies see Bluebird square off against Kite, December 5, 2015).

Clinical CAR-T studies in multiple myeloma and acute myelogenous leukaemia (AML; excluding China)

Source: EP Vantage, Clinicaltrials.gov

Project name	Antigen	Company	Academic centre	Indication(s)	Enrolment	Trial ID	Trial status
MB-102	CD123	Mustang (Fortress Bio)	City of Hope Medical Center	AML	30	NCT02159495	recruiting
Anti-CD 123 CAR	CD123		University of Pennsylvania	AML	15	NCT02623582	recruiting
bb2121	BCMA	Bluebird Bio & Celgene		multiple myeloma	50	NCT02658929	recruiting
CART-BCMA	BCMA	Novartis	University of Pennsylvania	multiple myeloma	30	NCT02546167	recruiting
Anti-BCMA CAR	BCMA		NCI	multiple myeloma	38	NCT02215967	recruiting
CTL019	CD19	Novartis	University of Pennsylvania	multiple myeloma	13	NCT02135406	active, not recruiting
Anti-LeY CAR	Lewis Y		Peter MacCallum Cancer Centre, Australia	myeloma, AML, MDS	6	NCT01716364	unknown

Meanwhile AML, given its extremely intractable nature, is a particularly attractive target, but there has so far been relatively little clinical work against it. One of the most interesting targets to watch will be CD123, with Western groups studying this antigen including Mustang (Fortress Bio). CD123 is also now the lead target for Cellectis's allogeneic CAR-T technology, though progress has been slow and UCART123 has yet to enter the clinic.

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Solid tumours - the big prize... and hardest of all

However remarkable initial response rates to CAR-T therapy continue to be in certain haematological cancers it is a different story in solid tumours, which for several reasons remain an extremely tough nut to crack. It is solid tumours that represent the big prize in CAR-T – US solid cancer incidence outnumbers by 10 to one that of haematological malignancies, where CAR-T competition is intense. However, none other than the NCI's Dr Steven Rosenberg, a pioneer of adoptive cell therapy whose work has focused on tumour-infiltrating lymphocytes rather than engineered T cells, has been quoted as saying that, with very few exceptions, CAR-T is likely to hold little promise beyond haematological cancers.

Indeed, early data suggest that there is still a huge amount of work to be done in solid tumours. For instance, the 2015 AACR meeting saw one of the first solid tumour readouts, with Novartis and the University of Pennsylvania's CART-meso, which failed to generate any responses among heavily pretreated patients with mesothelioma, ovarian cancer or pancreatic cancer, and, more worryingly, reported poor persistence of the CAR-T cells (AACR – Solid tumour CAR-T foray lives up to its low-key billing, April 20, 2015).

This, however, could be down to a poor target: mesothelin is overexpressed in some cancers but is also present on normal mesothelial cells. It is likely that there is no therapeutic window, as it seems not to be possible to give a dose high enough to generate anticancer activity without also stimulating severe off-tumour toxicity.

One of the biggest problems of solid tumours is that they tend to surround themselves with a hostile, immunosuppressive microenvironment. CAR-T cells are inefficiently trafficked to solid tumours, and the microenvironment, where multiple inhibitory factors are present, damps down T-cell function. It seems clear that a standard CAR-T approach will not cut it here, and groups associated with Juno and Novartis are leading the way in constructs that either have a novel design or that incorporate extra elements to boost activity in this setting, though all are at the preclinical stage at present.

A more fundamental issue is that solid cancers generally lack tumour-specific cell-surface antigens that can be targeted with CAR-T therapy – a fact that was already evident with the multiple failures of therapeutic cancer vaccines. In CAR-T specifically, as well as the CART-meso disappointment there was an earlier NCI trial of a Her2-directed therapy in which one patient died – likely because this antigen is also expressed on lung endothelium – causing a suspension of Her2-directed CAR-T studies.

But this has not stopped work on either antigen. Aurora Biopharma is still targeting Her2 with Baylor College, though when studies restarted after the patient death it was at what Baylor's Dr Stephen Gottschalk called a "homeopathic dose". A related approach – targeting ErbB dimers – is being pursued by Leucid Bio with a CAR project it calls LEU-001 (T1E28z), and early data have shown promising hints. One key here seems to be intratumoural delivery, which results in the T cells remaining fairly localised and thus avoiding adverse events in the lung.

Leucid Bio, a spin-out of the UK's King's College, started a small clinical study of T1E28z against head and neck cancer at Guy's Hospital. The group's chief scientific officer is Dr John Maher, who in the 1990s worked in Dr Michel Sadelain's lab at Memorial Sloan Kettering (Dr Sadelain is a scientific founder of Juno), and his focus is specifically on solid tumours, though he accepts that this represents "a huge leap".

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"It's pretty difficult to find a solid tumour that you cannot target with this CAR, at least in vitro," says Dr Maher. "But the obvious elephant in the room is how you can ever conceive of doing this safely." Apart from the targeting moiety this second-generation construct is the same as that used in Juno's JCAR015. There is another clever aspect that differentiates T1E28z from others: the addition to the CAR-T cells of a receptor that renders them responsive to the cytokine IL-4. This means that only the genetically engineered cells will grow during the manufacturing process on exposure to IL-4, enabling Dr Maher's team to obtain good transduction rates even in lymphopenic patients – those with extremely low T-cell counts.

> "The obvious elephant in the room is how you can ever conceive of doing this safely"

"It's very, very difficult to identify safe targets," says Dr Maher. "We need to be thinking about developing strategies to identify therapeutic windows, treading a fine line between a target that's upregulated on a tumour but expressed at lower levels on healthy tissue." (Interview – Leucid Bio swings for the fences in CAR-T, December 15, 2015).

Clinical studies of CAR-T therapies in solid tumours (excluding China, including completed trials)

Source: EP Vantage, Clinicaltrials.gov

Project name	Antigen	Company	Academic centre	Indication(s)	Enrolment	Trial ID	Trial status	Added feature(s)
anti-CEA MFEz	CEA	Cellular Therapeutics Ltd	Cancer Research UK	solid tumours	14	NCT01212887	terminated	–
anti-CEA CAR	CEA	–	Roger Williams Medical Center	adenocarcino-mas	48	NCT01723306	active, not recruiting	–
anti-CEA CAR	CEA	–	Roger Williams Medical Center	liver metastases	6	NCT02416466	recruiting	combination with Sir-Spheres
anti-CEA CAR	CEA	–	Roger Williams Medical Center	various	9	NCT00004178	completed	–
RNA cMet CAR	c-Met	–	University of Pennsylvania	breast cancer	15	NCT01837602	recruiting	–
EGFRvIII CAR	EGFRvIII	–	Duke University Medical Center	glioblastoma	48	NCT02664363	not yet recruiting	–
Anti-EGFRvIII CAR	EGFRvIII	Kite Pharma	NCI	malignant gliomas	107	NCT01454596	recruiting	–
CART-EGFRvIII	EGFRvIII	Novartis	University of Pennsylvania	glioblastoma	12	NCT02209376	recruiting	–
CART-EGFRvIII	EGFRvIII	Novartis	University of Pennsylvania	glioblastoma	8	NCT02666248	enrolling by invitation	–
LEU-001 (T1E28z)	ErbB dimers	Leucid Bio	King's College London	head and neck cancer	30	NCT01818323	recruiting	IL4 receptor to aid expansion
FAP-specific T Cells	FAP	–	University of Zurich	mesothelioma	6	NCT01722149	recruiting	CD8+ T cells
allogeneic lymphocytes	FRα	–	NCI	ovarian cancer	14	NCT00019136	completed	–

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Project name	Antigen	Company	Academic centre	Indication(s)	Enrolment	Trial ID	Trial status	Added feature(s)
GD2 CAR	GD2	–	Baylor College & NCI	neuroblastoma	11	NCT01822652	active, not recruiting	–
VZV-specific GD2 CAR	GD2	–	Baylor College & NCI	osteosarcoma	26	NCT01953900	recruiting	–
GINAKIT Cells	GD2	–	Baylor College of Medicine	neuroblastoma	18	NCT02439788	not yet recruiting	uses natural killer T cells
EBV-specific GD2.CAR	GD2	–	Baylor College of Medicine	neuroblastoma	19	NCT00085930	active, not recruiting	–
EBV-specific GD2.CAR	GD2	–	Children's Mercy Hospital Kansas City	neuroblastoma	5	NCT01460901	completed	–
GD2 CAR	GD2	–	NCI	GD2+ve solid tumours	72	NCT02107963	recruiting	–
1RG-CART	GD2	Autolus	University College, London	neuroblastoma	27	NCT02761915	recruiting	–
AU105	Her2	Aurora	Baylor College of Medicine	glioblastoma	14	NCT02442297	not yet recruiting	–
AU105	Her2	Aurora	Baylor College of Medicine	glioblastoma	16	NCT01109095	active, not recruiting	–
AU105	Her2	Aurora	Baylor College of Medicine	sarcoma	36	NCT00902044	recruiting	–
HER2.CAR VSTs	Her2	–	Baylor College of Medicine	various	19	NCT00889954	active, not recruiting	EBV T cells
Her-2 CAR	Her2	–	NCI	various	1	NCT00924287	terminated	–
MB-101	IL13Rα2	Mustang (Fortress Bio)	City of Hope Medical Center	glioma	36	NCT02208362	recruiting	–
GRm13Z40-2	IL13Rα2	–	City of Hope Medical Center	glioma	6	NCT01082926	completed	CD8+ T cells
anti-IL13 zetakine	IL13Rα2	–	City of Hope Medical Center & NCI	glioma	3	NCT00730613	completed	CD8+ T cells
CE7R	L1CAM (=CD171)	–	Fred Hutchinson & NCI	neuroblastoma	10	NCT00006480	completed	CD8+ T cells
JCAR023	L1CAM (=CD171)	Juno	Seattle Children's Hospital	neuroblastoma	80	NCT02311621	recruiting	–
iCasp9M28z	Mesothelin	–	Memorial Sloan Kettering	various	24	NCT02414269	recruiting	–
mesothelin CAR	mesothelin	–	NCI	various	136	NCT01583686	recruiting	–
RNA Meso-CIR T	Mesothelin	–	University of Pennsylvania	mesothelioma	18	NCT01355965	completed	–
RNA mesothelin SS1	mesothelin	–	University of Pennsylvania	pancreatic cancer	10	NCT01897415	completed	–
CART-meso	Mesothelin	Novartis	University of Pennsylvania	various	21	NCT02159716	active, not recruiting	–
CART-meso	mesothelin	Novartis	University of Pennsylvania	various	50	NCT02388828	recruiting	–
CART-meso + CTL019	mesothelin + CD19	Novartis	University of Pennsylvania	pancreatic cancer	12	NCT02465983	active, not recruiting	–
JCAR020	MUC16	Juno	Memorial Sloan Kettering	gynaecological & peritoneal cancers	30	NCT02498912	recruiting	IL12-secreting "armored CAR"

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Project name	Antigen	Company	Academic centre	Indication(s)	Enrolment	Trial ID	Trial status	Added feature(s)
BPX-601	PSCA	Bellicum	–	pancreatic cancer	30	NCT02744287	not yet recruiting	GoCAR - inducible MyD88/ CD40 domain
autologous T cells	PSMA	–	Memorial Sloan Kettering	prostate cancer	18	NCT01140373	recruiting	–
anti-PSMA CAR	PSMA	–	Roger Williams Medical Center	prostate cancer	12	NCT01929239	active, not recruiting	–
JCAR024	ROR1	Juno	Fred Hutchinson Cancer Center	various	60	NCT02706392	not yet recruiting	might be CD4+:CD8+ defined composition
VEGFR2 CAR	VEGFR2	–	NCI	various	24	NCT01218867	completed	–

Other groups are continuing to pursue solid tumours with CAR-T approaches: as well as Novartis's CART-meso the NCI's GS2 CAR-T disappointed in neuroblastoma. Juno is running a neuroblastoma trial with JCAR023, targeting L1-CAM, while Bellicum plans to go into pancreatic cancer with BPX-601, a CAR-T against PCSA whose phase I trial has yet to begin enrolment.

Juno recently began a trial of JCAR024, an anti-Ror1 CAR-T project that it says will need to incorporate additional features to improve T-cell function in an immunosuppressive microenvironment to overcome resistance to TGF-beta signalling, or adenosine pathway or PD-1-mediated suppression. Juno's Mr Levitsky, who at the AACR meeting presented preclinical data with JCAR024, said some of these "bells and whistles… would be built into [JCAR024] only once we have an assessment of safety". This fits its phase I plan, which initially targets CLL and mantle cell lymphoma – indications that the science chief calls mere proof of concept. "The real motivation of course is the diseases that CD19 is not on," he states, stressing that a second cohort will look at Ror1-positive triple-negative breast and lung cancers. Another issue is that the advanced CAR constructs still use murine antigen-binding domains, though all three leading companies are pursuing humanised or fully human binders. The current version of Juno's JCAR024 uses a rabbit-derived anti-Ror1 domain, and Mr Levitsky says the group is in lead selection for a fully human binder.

Novartis and Penn are working on a CAR-T against EGFRvIII, preliminary clinical data on which were also presented at this year's AACR meeting. The therapy appeared to be safe and well tolerated in nine advanced glioblastoma subjects infused, though it was too early to assess clinical activity. Kite, which has rights to the NCI's anti-EGFRvIII CAR, recently downgraded this to preclinical status, saying it was looking into "additional engineering to improve the product candidate". The NCI's clinical study of this project has been suspended as a result of an internal review into certain labs that has put a halt on all new patient dosing.

Other groups want to make the most of the few remaining amenable targets. Juno's study with a CAR against Muc16, for instance, is the group's first attempt at taking an "armored" CAR into the clinic. In this case the T cells are additionally made to express the cytokine IL-12, which it is hoped will induce T-cell response, enhance expansion and perhaps even overcome inhibition mediated by T regulatory cells. Some groups go as far as to suggest that it will ultimately be impossible to target solid tumours without also giving IL-12.

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But Dr Roisin O'Cearbhaill, the primary investigator of the anti-Muc16 trial, cautions that inducing IL-12 release could have toxic effects on the lung, liver and intestine, possibly causing death. MSKCC's Dr Renier Brentjens, a scientific founder of Juno, has proposed two further "armored" CARs: CAR-T cells that additionally express the CD40 ligand, and those that deliver checkpoint blockade (CAR-T meeting – To hit solid tumours use a superCAR, March 14, 2016).

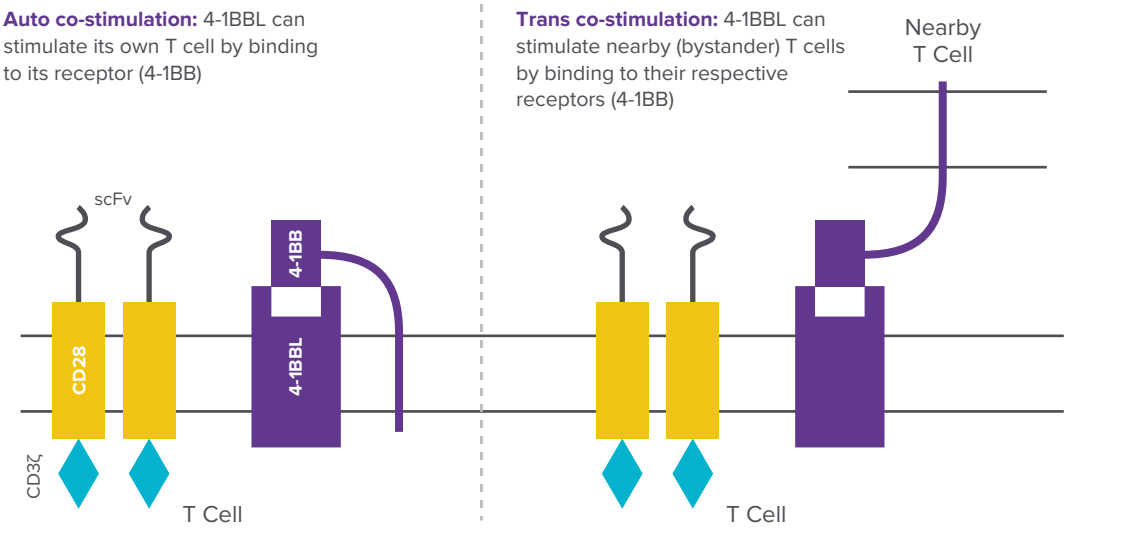
Optimisation of existing CAR constructs and additions of various new features into them will likely be a key focus for upcoming work. Dr Steven Albelda of the University of Pennsylvania is working on a chimaeric switch receptor that could boost the efficacy of CAR-T cells in solid tumours by converting a negative PD-1 signal into a positive one. He has also presented a CAR designed additionally to express the protein RIAD. This construct could make T cells resistant to the immunosuppressive effects of adenosine and PGE2 – the two most powerful factors in the tumour microenvironment.

Dr Sadelain, whose work at Memorial Sloan Kettering is licensed to Juno, has spoken extensively about the need to improve on the current second-generation CAR constructs. Interestingly, he has expressed reservations about third-generation constructs (these incorporate not one but two co-stimulatory domains in the CAR construct in addition to CD3zeta), an example of which is in clinical development at Sweden's Uppsala University and might be licensed to Bluebird Bio. Instead, he is putting his faith in a fourth-generation construct, in which a 4-1BB ligand is expressed on the T cell – separately from the CAR construct.

The idea here is for the separate 4-1BBL domain to promote trans-costimulation – acting as an agonist to stimulate either its own T cell or to co-stimulate bystander T cells. Dr Sadelain's aim is to take this into the clinic in 2016.

A proposed 4th-generation construct that could enter the clinic in 2016

Source: Michel Sadelain – AACR 2016



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But there are problems

Lack of persistence

A big problem for all CAR-T projects is lack of persistence. Even in ALL studies reporting remissions of 90% or more, many patients relapse within a year. Relapses are most common in ALL, but also occur in lymphoma studies: in Novartis's CTL019 trial two partial remissions turned to progressive disease in six to 12 months, while in Kite's seven-patient Zuma-1 trial of KTE-C19 one complete remission relapsed in three months, as reported at ASH 2015.

Improving the design of CAR-T therapies

It is still unclear what might be the cause of poor persistence, and how this could be remedied. One idea is that it depends on the co-stimulatory domain used in the CAR constructs: a developing view is that CD28 is a more potent activator of T cells than 4-1BB, but 4-1BB is associated with longer cell persistence. This theory has yet to play out, but will have to be watched especially by followers of commercial entities that are already wedded to one approach or the other, and thus have no way of changing tack easily. Moreover, use of new co-stimulatory domains (ICOS, CD40, MyD88) is being investigated, as is that of third-generation constructs that employ more than one on the same construct.

It will also be important to compare the properties of the different transfection methods being used. The most popular are gamma-retroviral and lentiviral, and there is some evidence to suggest that gamma-retroviruses risk causing gene silencing, meaning that lentiviral transfection could be associated with better persistence, but again it is too early to say for sure. "Solely based on the data that's published right now, I'd say there's more compelling data that CD28 versus 4-1BB is a bigger part of the [persistence] equation than lentiviral versus gamma-retroviral," Penn's Dr Stephan Grupp has speculated. "But that is just a guess" (Therapy focus – How do you solve a problem like CAR-T relapse? December 22, 2015).

Two other methods of getting genetic material into the T cells need to be borne in mind: MD Anderson's Sleeping Beauty virus-free transposon/transposase DNA plasmid-based system, and mRNA electroporation, an approach being pioneered by MaxCyte and Penn.

Sleeping Beauty's originator, MD Anderson, yielded a $115m deal with Ziopharm Oncology and Intrexon last year, though based on the data generated it seemed clear that this was a case of corporate laggards betting on a technology that had failed to generate a great deal of excitement (Sleeping Beauty wakes up to $115m deal, January 14, 2015).

MD Anderson's Dr Partow Kebriaei has presented Sleeping Beauty CAR-T studies at several ASH conferences, highlighting safety, in particular the lack of cytokine release syndrome, and possible cost advantages. However, the relative safety of this approach could be due to it simply not being very efficacious, as well as the fact that in post-transplant patients – the setting in which many of Dr Kebriaei's studies were done – tumour burden is low. Serious adverse events of cytokine release and neurotoxicity both correlate with disease burden and response.

Dr Kebriaei broadly accepts that the CAR construct could be improved on, for instance by changing its co-stimulatory domain from CD28 to 4-1BB to improve T-cell persistence. Results from studies using a 4-1BB co-stimulatory domain are keenly awaited.

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Humanising binding domains

All that said, perhaps the most obvious feature of the initial wave of CAR-T projects that might be contributing to poor persistence is their use of murine ScFv (antigen-binding) regions. The theory is that the murine nature of this element is causing the T cells to be rejected by the human host, and this has led to recent moves into developing CAR constructs with humanised or fully human binding domains.

Kite, for instance, signed a new co-operative R&D agreement with the NCI – specifically with Dr James Kochenderfer – to develop a fully human anti-CD19 CAR-T candidate for leukaemias and B-cell lymphomas, and this is now in a clinical trial. Juno, meanwhile, has gone a step further, striking a full licence to a fully human binding region developed by a little-known private biotech, Eureka Therapeutics. The initial focus of the deal is a MUC16 binder that will be used in conjunction with JCAR020, Juno's anti-MUC16 CAR-T project, which recently entered a clinical trial in ovarian, fallopian tube and primary peritoneal cancers.

This binding domain was developed by Eureka and Memorial Sloan Kettering Cancer Center, which is Juno's partner on the CD19-directed JCAR015 project. A statement by Mark Frohlich, Juno's head of development and portfolio strategy, cites the company's continued pursuit of fully human binding domains, "with the goal of optimizing cell persistence" (Juno and Kite follow Novartis to make CAR-T human, January 8, 2016). JCAR021, Juno's fully human anti-CD19 construct, is in preclinical development.

Juno previously said it had access to a library of fully human scFv domains, and separately its anti-CD22 CAR-T project, JCAR018, licensed from the NCI via Opus Bio, uses a fully human binding domain. Apart from that the only clinical results so far have come from Novartis's humanised anti-CD19 construct, CTL119. There is little data to go on at present, but one of the most hotly awaited CAR-T presentations of the 2016 Asco meeting concerns data from a trial of eight CTL019-treated patients retreated with CTL119. There have been four responses, with one of the patients in complete remission for seven months. Dr Shannon Maude, of the Children's Hospital of Philadelphia, reports that two of the four responding patients were previously resistant to reinfusion of the murine CTL019. It is early days, but further positive data in a larger patient set would put Novartis in a quandary: should the group even bother filing CTL019, or should it just switch to CTL119? (Therapy focus – How do you solve a problem like CAR-T relapse?, December 22, 2015). At AACR Dr Sadelain put it bluntly, saying future CAR-T work "should now only make use of human binding domains".

Defined cell composition

Recently Juno has been putting its faith in what it calls a defined-composition CAR-T product, based on the work of Dr Stanley Riddell at the Fred Hutchinson Cancer Center. This introduces additional cell-sorting steps in the manufacturing process to isolate the specific T-cell subtype(s) that are to be transduced, expanded and reinfused into the patient, rather than simply applying these processes to bulk T cells.

The goal is to improve peak concentration, persistence and thus efficacy of the CAR-T cells, and a recently published paper has demonstrated the practical benefits of this approach in patients; Juno's confidence now stretches to having its second planned anti-CD19 CAR-T project, JCAR017, use cells of defined composition. The current thinking is that a product comprising a 50:50 mixture of CD4+ and CD8+ T cells removes cell populations that might inhibit transduction and in vitro expansion, as well as providing a product that is more homogeneous and thus yields more reproducible behaviour.

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Data presented at AACR from the Fred Hutchinson study were impressive: a 93% complete remission rate in adults with ALL. Dr Riddell has also presented data with a defined-composition product in refractory non-Hodgkin's lymphoma, showing 50% complete remission and 69% overall response rates in lymphodepleted patients across all doses. Perhaps the most important finding was that this defined-composition product allowed a reduction in cell doses, yielding efficacy comparable to previous studies with bulk cells, and importantly with reduced toxicity. For instance, in adult ALL rates of severe cytokine release and neurotoxicity were a relatively low 23% and 52% respectively, while in lymphoma they were 20% and 35% respectively.

One important conclusion, therefore, is that CAR-T cells of uniform composition reveal a dose/toxicity relationship, allowing toxicity to be reduced in patients with high tumour burden by adjusting down the dose without compromising the response rate. That said, the additional manipulation that has to be done with the cells necessarily increases complexity and cost even further beyond what is already perceived as being very expensive.

More fundamentally, work is needed to improve further on the current CAR constructs, and Dr Riddell told the AACR meeting: "They might still not be optimal as regards signalling." Two important studies are specifically designed to test one approach versus another in a clinical setting: Jae Park's NCT00466531 at Memorial Sloan Kettering compares an anti-CD19 CAR with a CD28 co-stimulatory domain versus one with a 4-1BB domain, as well as lentiviral versus gamma-retroviral transduction and the different lymphodepleting regimens used.

And the Trident trial, which has yet to get under way, will compare the following three anti-CD19 CAR constructs that all comprise a CD28 co-stimulatory domain: one with a separate 4-1BBL element, one that separately secretes IL-12, and one with a separate CD40L domain.

Relapses through antigen escape

The above problem of poor cell persistence leads to so-called antigen-positive relapses (the target cells still retain the relevant antigen, and relapse results from waning CAR-T cells). A separate issue is a so-called antigen-negative relapse, also known as antigen escape, whereby the CAR-T therapy is no longer able to target the desired cell type because these cells no longer display the desired antigen.

These escape mechanisms have so far only been detected in CD19-targeting therapies, but the problem could well apply to other antigens, too. On the other hand, there could be something about CD19 that makes it a particularly unstable antigen.

There are two documented types of antigen escape: antigen loss and lineage switching, and these necessitate very different means by which relapsing patients might be retreated.

Antigen loss

"Loss" of the CD19 antigen is actually a misnomer, and current thinking is that this involves just part of the CD19 protein being spliced out, as a response to CD19-directed treatment. The subsequent proliferation of B cells carrying these CD19 splice variants yields a growing population of cells that can no longer be targeted with a CD19 CAR because they no longer display the relevant epitope.

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One answer to this kind of relapse could be to retreat the patient with a CD22-directed CAR. CD22 is another antigen present on B-cell lineages, and this leads on to Juno's JCAR018, an anti-CD22 CAR derived from work at the NIH that Juno bought from Opus Bio in 2014 for about $84m. At ASH 2015 the NCI's Dr Daniel Lee said he was continuing to enrol CD19-escaped patients into a CD22 CAR-T study, though all the data and IP arising from this presumably belong not to the NCI's CRADA partner Kite, but to Juno.

At the 2016 AACR meeting the NCI's Dr Terry Fry presented new data on three additional young adult ALL patients who had been given a high dose of CD22 anti-CAR-T cells: all three had complete responses that were ongoing at three to six months. One of six earlier lower-dose patients had also had a complete response, but relapsed after three months. This appeared to be the first sign of real efficacy with a CD22 CAR, and another key finding concerned the two most important side effects of adoptive cell therapy: while cytokine release was seen, there was no neurotoxicity.

Juno is paying for elements of Dr Fry's study while remaining slightly at arm's length, its chief scientific officer, Hy Levitsky, told *EP Vantage*, adding: "We will of course be running our own studies [of JCAR018], under our own INDs. Our plans are very much aligned."

He called the three new complete responses a "very nice signal", and said the lack of neurotoxicity was intriguing, but cautioned that these were still very small patient numbers. Still, it is well worth noting that one of the three patients responded after a CD19-negative relapse to an anti-CD19 CAR. For the other two it was their first CAR-T treatment. These early data therefore support use of an anti-CD22 CAR-T therapy both in relapsed and in anti-CD19 CAR-T-naive patients.

At a time when the cost of CAR-T already looks stretched a retreatment strategy looks hard to sustain economically, but treating CAR-T-naive patients with a CD22-directed construct offers an intriguing prospect. And, while CD19-directed therapies abound, Juno's JCAR018 is one of only two commercially owned CD22-directed CARs in the clinic.

Anti-CD22 CAR-T projects in the clinic (excluding China)

Source: EP Vantage, Clinicaltrials.gov

Project name	Company	Academic centre	Indication(s)	Enrolment	Trial ID
JCAR018	Juno, via Opus Bio	NCI	B-cell malignancies	57	NCT02315612
CART22 cells	Novartis	University of Pennsylvania	ALL	15	NCT02588456
CART22 cells	Novartis	University of Pennsylvania	ALL	15	NCT02650414

What is still not known is whether patients can suffer loss of the CD22 antigen as a response to treatment, but Dr Fry went as far as suggesting that treating patients with an anti-CD22 CAR could prevent relapses by CD19 antigen loss (The next CAR-T target generates promise and caution, April 25, 2016).

Dr Lee also separately cites a planned study of a bivalent CD19-CD22 CAR, a highly unusual single CAR construct that was featured in a poster at ASH 2015. The NCI authors concluded that the order of the CD19 and CD22 binding domains and the length of the linker affected function, and despite some evidence of activity further optimisation is needed before this enters the clinic.

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Lineage switching

This second CD19-negative relapse mechanism is potentially more concerning. Dr Fry points to preclinical trials in which a B-cell malignancy treated with a CD19 CAR switches lineage, so the cells phenotypically stop being B cells, and become myeloid cells. Not only would any such patients no longer be candidates for either a CD19 or a CD22-directed therapy, they would now have a mixed-lineage leukaemia for doctors to deal with – a potentially serious development.

Juno's Mr Levitsky, however, is sanguine. "The outgrowth of the myeloid phenotype is concerning, but we've not yet seen it in adults," he stated. "Paediatric and adult ALL are very different diseases, and paediatric ALL has been curable with chemotherapy for decades."

He also expressed confidence in the anti-CD22 construct that Dr Fry had developed, confirming that it was the one Juno would take forward. This had undergone several alterations, including the addition of a spacer to move the binding site away from the cell membrane, and changing the co-stimulatory domain from CD28 to 4-1BB to improve persistence.

Lack of safety

Toxicity is a problem that has plagued adoptive cell therapies since they first started showing startling evidence of efficacy. In fact the two key adverse events – severe cytokine release syndrome (CRS) and neurotoxicity – are broadly correlated with efficacy. They are still a problem, though companies and hospitals are fast developing strategies to deal with this.

Cytokine Release Syndrome (CRS) with CART19 Therapy

Source: Dr Noelle Frey, University of Pennsylvania

Ref	Programme/CAR	Population	Response	CRS
Acute Lymphoblastic Leukemia				
Maude et al. NEJM 2014	PENN 4-1BB	N=30(ALL) Children & Adults	CR=90%	100% CRS 27% Severe
Davila et al. ScriTrMed 2014	MSK CD28	N=16(ALL) Adults	CR=88%	43% Severe
Lee et al. Lancet 2015	NCI CD28	N=21(ALL) Children & AYA	CR=67% Intent to Treat	76% CRS 28% Severe
Non-Hodgkin's Lymphoma & Chronic Lymphocytic Leukemia				
Kochenderfer JCO 2015	NCI CD28	N=15(NHL/CLL)	CR=53% PR=27%	27% Severe
Porter et al. SciTrMed 2014	PENN 4-1BB	N=14(CLL)	CR=29% PR=29%	42% Severe

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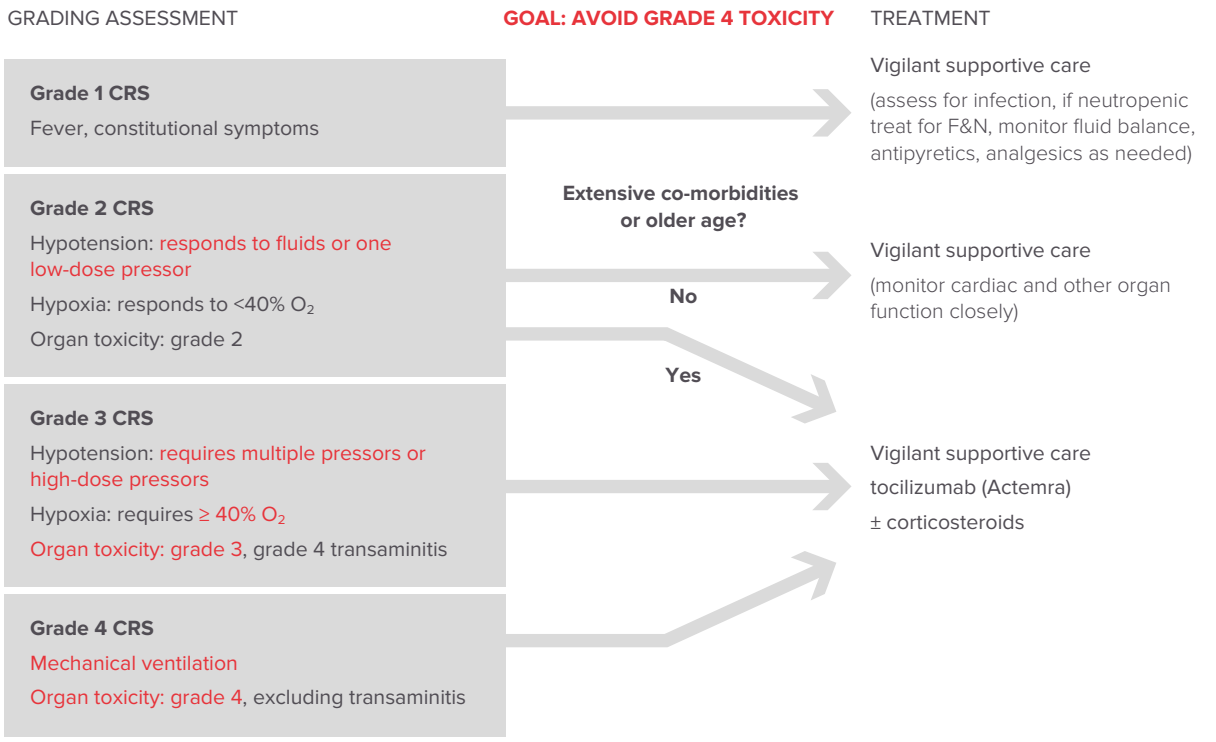
Practical strategies include more gradual increases in CAR-T dosing, and education of hospital staff in how to treat a patient experiencing one of these episodes. One development has been very specific classifications to grade the severity of an episode, and treatment strategies for each, one result being a flow chart proposed by the NCI's Dr Daniel Lee.

Treatment algorithm for management of CRS

Source: Lee DW, et al. Blood 124(2):188-195, 2014



The Fred Hutchinson Cancer Research Center's Dr Cameron Turtle has suggested that toxicity might be related to the lymphodepleting regimen patients received before CAR-T cell infusion. Severe cytokine release and neurotoxicity were more common in patients depleted using cyclophosphamide/fludarabine than non-Cy/Flu regimens in his study of JCAR017. On the other hand, Cy/Flu lymphodepletion was associated with higher remission rates. Lymphodepletion – the destruction of a person's existing T cells – aids cell engraftment and is necessary to boost efficacy of adoptive cell therapy.

More positive news came from the NCI's study of an anti-CD19 CAR-T in which a 60% complete remission rate was accompanied by just 15% severe cytokine release, thanks to the use of Dr Lee's grading assessment/treatment flowchart. Dr Lee and Memorial Sloan Kettering's Dr Kevin Curran are two physicians who are putting their faith in these treatment algorithms, which they hope will turn cytokine release syndrome into something that is manageable thanks to its understanding and grading, and to the development of appropriate treatment approaches.

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Dr Lee says the keys are to know the potential for risk before treatment, and to observe patients closely and vigilantly, administering anti-cytokine therapy – Actemra and steroids – when necessary. Some patients need multiple interventions, and a vigilant eye must be kept on neurotoxicity even after cytokine release resolves. He also stressed the importance of extensively educating intensive care staff and nurses.

Suicide genes

Fears about adverse events have driven several groups to develop so-called suicide genes that can be incorporated into the CAR-T cells to allow them to be destroyed, usually on addition of a separate molecular "trigger", in the event of serious toxicity. Indeed, at one extreme there exists a belief that no CAR-T project will gain FDA approval without an inbuilt switch to ablate the T cells quickly in an emergency.

Perhaps the smartest example is inducible caspase 9 (iC9) developed at Baylor College of Medicine and licensed to Bellicum; this incorporates intracellular caspase domains that dimerise upon infusion of the small molecule rimiducid, leading to cell destruction. This at present is seen as the cleanest suicide switch, involving simple apoptosis, and has been demonstrated in humans, albeit in the setting of stem cell transplantation, and not in a Bellicum study but in an earlier trial run by Baylor.

Meanwhile, Juno and Cellectis incorporate expression of cell surface proteins – a truncated EGF receptor and RQR8 – that can be triggered with Erbitux and Rituxan respectively to cause T-cell ablation. However, these kill via antibody-dependent cell-mediated cytotoxicity – a process that causes inflammation – as opposed to Bellicum's clean apoptosis.

This is an important difference, as demonstrated by the fact that doctors running studies of Juno constructs that include EGFRt have not dared use it for fear of the resulting inflammatory response. In fact, many no longer refer to EGFRt as a suicide switch and instead say this protein is expressed on cells to aid in their selection during manufacturing. Fred Hutchinson's Dr Cameron Turtle put it bluntly: "It's a nice idea," he told the CAR-T seminar at Memorial Sloan Kettering in March, "But I'm nervous about [infusing] Erbitux."

Bellicum itself insists that the only true suicide switch is the one in its possession (Interview – Bellicum takes on the suicide switch wannabes, November 20, 2015). Interestingly the caspase suicide gene has also been incorporated into several constructs being studied by Bellicum's competitors, including Memorial Sloan Kettering, though given the IP situation this could not proceed beyond academia into a commercial application outside Bellicum.

Given the paucity of data it is by no means clear how efficient these off-switches are; how quickly can they ablate the T cells? What toxicities can they reverse? It has been suggested, for instance, that severe cytokine release can be stopped, but if a patient is experiencing neurotoxicity it probably matters little even if at that point all the T cells can be killed. Furthermore, even Bellicum admits that after ablation a population of T cells seems to remain that goes on to re-expand. As to whether you have to have the ability to get rid of every last T cell, "that's a question we fundamentally have to answer in the clinic", says its chief executive, Tom Farrell.

Bellicum is also working on a related approach designed to achieve the opposite effect – a rimiducid-regulated switch to activate the T cells in vivo rather than switching them off. This comprises a first-generation CAR-T construct that includes a separate, MyD88/CD40 section that can perform the co-stimulatory function only once caspase dimerisation has been triggered by rimiducid. This can be achieved in stepwise fashion to achieve something akin to dose titration. A phase I pancreatic cancer study of BPX-601, the first project to include this, is to start enrolling in June.

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Bellicum has previously floated the idea of licensing its suicide gene technology to other CAR-T players on an antigen-by-antigen basis for targets that it has no desire to develop itself (A CAR-T suicide switch for hire, March 12, 2015).

In fact numerous ablation technologies have been attempted in the past, but the inducible caspase and (to the limited extent that this still represents a feasible strategy) the truncated EGFR approaches are the only ones with a significant presence in clinical trials. Other approaches have included HSV-TK (Fred Hutchinson Cancer Center), HyTK and truncated CD19 (both City of Hope). Merck KGaA, in the development of MD Anderson's CAR-T assets through its deal with Intrexon and Ziopharm, intends to make use of Intrexon's Rheoswitch gene regulation technology. However, this has not yet been tested clinically in a CAR construct.

Projects incorporating suicide genes (excluding China)

Source: EP Vantage and company filings

Suicide gene	Academic centre	Company	Project name	Antigen
EGFRt	City of Hope Medical Center	Mustang (Fortress Bio)	MB-102	CD123
EGFRt	Fred Hutchinson & NCI	Juno	JCAR014	CD19
EGFRt	Seattle Children's Hospital	Juno	JCAR017	CD19
EGFRt	City of Hope Medical Center & NCI	–	CD19CAR	CD19
EGFRt	Fred Hutchinson & NCI	–	CMV or EBV-specific CD19 CAR	CD19
EGFRt	Seattle Children's Hospital	Juno	JCAR023	L1CAM (=CD171)
EGFRt	Memorial Sloan Kettering	Juno	JCAR020	MUC16
EGFRt	Fred Hutchinson Cancer Center	Juno	JCAR024	ROR1
HSV-TK	Memorial Sloan Kettering	–	autologous T cells	PSMA
HyTK	City of Hope Medical Center & NCI	–	CD19CAR	CD19
HyTK	City of Hope Medical Center & NCI	–	anti-IL13 zetakine	IL13Rα2
HyTK	Fred Hutchinson & NCI	–	CE7R	L1CAM (=CD171)
iC9	Baylor College & NCI	–	VZV-specific GD2 CAR	GD2
iC9	Baylor College of Medicine	–	GINAKIT Cells	GD2
iC9	NCI	–	GD2 CAR	GD2
iC9	Memorial Sloan Kettering	–	iCasp9M28z	mesothelin
RQR8	University College, London	Cellectis, Pfizer & Servier	UCART19	CD19
suicide gene cassette	University College, London	Autolus	1RG-CART	GD2
truncated CD19	City of Hope Medical Center	Mustang (Fortress Bio)	MB-101	IL13Rα2

There are other ways of improving CAR-T safety, and this is the subject of work at groups including Juno, which has spoken of dual CAR constructs for use where an antigen is present on healthy as well as cancer cells. The idea here is for one CAR to respond to the desired antigen in the normal stimulatory fashion but for the second CAR to send an inhibitory signal if a second antigen, specific to healthy tissue, is also present.

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A similar approach is being pursued by Dr Martin Pule at University College, London, who is also a scientific founder of the private UK CAR-T company Autolus. Dr Pule has researched Boolean logic-gated CAR-T cells expressing two constructs, A and B, presenting the following possible permutations:

1) A OR B. Sends a stimulatory signal when either of the two antigens is present; this could make the T cells resistant to the escape of either of the two antigens, for instance.

2) A AND NOT B. Sends a stimulatory signal when antigen A is present, but not when antigen B is also present. This could be used if antigen A is present on the tumour, but B is present only on healthy cells (similar to the Juno example above).

3) A AND B. Sends a stimulatory signal only when both antigens are present. This could be used if both antigens are expressed on the tumour, but are also seen individually on healthy cells.

4) B AND NOT A. Sends a stimulatory signal when antigen B is present, but not when antigen A is also present.

All of these approaches, as well as the Juno inhibitory dual CAR concept, are in early stages of research. UCL recently started a clinical trial of an anti-GD2 CAR in neuroblastoma, and it is likely that this is an Autolus project; Dr Pule's group had previously published research on a next-generation anti-GD2 CAR with a "suicide gene cassette".

Separately, the UK's Leucid Bio is experimenting with intratumoural delivery, which it says has decreased off-tumour toxicity as the CAR-T cells remain fairly localised. Also these patients are not being lymphodepleted in the hope of avoiding toxicity through uncontrolled expansion and sustained engraftment of CAR-T cells (see solid tumour section). However, this is still a fairly niche approach.

Other safety concerns

Beyond CRS and neurotoxicity there are other safety concerns, classified by Massachusetts General Hospital's Dr Marcela Maus into several broad groupings:

Typical CAR-T toxicities

Source: EP Vantage

Description
on-target, off-tumour
off-target cross-reactivity with unintended antigens
effects on bystander innate cells causing systemic cytokine release syndrome (CRS)
allergy
graft-versus-host disease (GvHD)
integration site oncogenesis
replication-competent virus

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The solutions to some of these problems are easy in theory but difficult in practice. For instance on-target/off-tumour effects can be eliminated by finding the right target, though purely tumour-restricted targets are few and far between. Prior experience with naked antibodies can hint at what to expect, but can give false negatives, while prior toxicity with an antibody-drug conjugate could be a false positive, Dr Maus reckons.

Screening for off-target cross-reactivity is very difficult preclinically, but fortunately this toxicity is relatively rare. Likewise, allergy has only been seen once, on repeated exposure to a CAR with a murine-derived antigen-binding region. Graft versus host disease (GvHD) comes into play with allogeneic products (see below).

Integration site oncogenesis – the insertion of genetic material causing disruption of oncogenes and prompting development of malignant cells – and replication-competent virus – the possibility of the retrovirus being used to transduce genetic material having viral activity – are extremely serious potential toxicities. They featured prominently in development of the first wave of gene therapies, but fortunately have not been encountered in CAR-T studies in the clinic. These risks could prove extremely cumbersome if they resulted in FDA-mandated action, such as the performance of an integration site analysis for every lot of cells produced, though at present there is no such requirement. Cell products and infused patients are routinely tested for presence of replication-competent virus.

In March 2016 the US FDA suggested the creation of two databases to monitor the safety of anti-CD19 CAR-T therapies across the various sponsors' ongoing clinical trials. This move seems to have been driven by the very small sizes of each of the studies, a fact that necessarily makes analysing safety very difficult. The pilot project aims to evaluate safety and manufacturing across INDs to inform and build risk-prediction and risk-mitigation models.

It is at present uncertain precisely what effect this might have on sponsors' CAR-T projects, but it seems a safe bet that this plan indicates a cautious approach that could add an extra obstacle to approval.

Manufacturing

Despite complexity and cost of manufacturing being publicly dismissed by many companies and investors as something that will be ironed out soon enough, this remains the single biggest stumbling block to the widespread adoption of CAR-T as a commercial product. A recent sellside comment is telling: in February Citi wrote that the CAR-T production process was much more streamlined and less costly than that used by Dendreon, and that logistics were less complex.

Dendreon's Provenge, a therapeutic cancer vaccine that was the first autologous immunotherapy to gain commercial approval in the West, was a spectacular flop partly because its manufacturing was too convoluted to be commercially viable.

In fact the main advantage that CAR-T has over Provenge is that T cells can be frozen for shipping, meaning that they require much less co-ordination between collection and administration than was the case with Provenge, which had to be shipped fresh. The actual CAR-T manufacturing procedure is more complex, takes many times longer and – at least going by the current methods used by academic labs – is severalfold more expensive than was the case with Dendreon. No doubt the disaster of Provenge is still fresh in many people's minds.

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According to current procedures a typical CAR-T manufacturing run (ie, one without additional cell sorting to generate a product containing a defined composition of a T-cell subtype) will comprise:

A typical CAR-T manufacturing process

Source: EP Vantage

Step	Description
1	Leukapheresis (separating out the white blood cells from a patient's blood) at a hospital
2	Transport to a dedicated laboratory
3	Selection and isolation of T cells
4	Viral transduction (to get into the cells the genetic material to express the CAR construct on their surface)
5	T-cell activation and expansion
6	T-cell washing
7	Cryopreservation
8	Transport back to the hospital
9	Reinfusion into the patient

This can take around two weeks in total. Clearly this needs to be improved on significantly, and automating many of the steps is a key focus. At this year's AACR meeting Kite Pharma's chief medical officer, David Chang, described a manufacturing process taking as little as six days, whereby leukapheresis, and cell enrichment and activation take place immediately, transduction takes two days, and the remaining four days comprise expansion, harvesting and storage. Meanwhile, Juno recently revealed that it was making progress with a manufacturing process that could take as little as two days. "To be clear, we are not there yet," cautioned the group's chief executive, Hans Bishop.

In the meantime, significant investments are being made. Kite says that after process improvements its newly constructed plant – strategically placed very close to Los Angeles airport – is on stream and producing KTE-C19 for clinical trials. As far as commercial supply goes, equipment installation will be completed in the first half of 2016, followed by FDA inspection after a planned BLA filing this year.

Juno is aiming to be producing clinical trial quantities of JCAR015 at its Bothell facility by the end of this quarter. Commercially it is neck and neck with Kite, and likewise will need to clear a pre-approval inspection. Novartis is focusing its US manufacturing efforts on a Morris Plains, New Jersey, plant it bought from Dendreon in 2012 for $43m. To embark on building manufacturing capacity well before approval is bullish – especially considering how far there is still to go before adoptive cell therapy can make the leap from a niche procedure into a product with broad commercial potential. Still, it is hard to expect anything else from companies that have raised hundreds of millions of dollars from public investors.

Production failures

Beyond the inherent complexity lie additional manufacturing problems: some patients have insufficient T cells to allow a product to be made; viral transduction is relatively inefficient; cell expansion is by its nature uncontrolled, meaning that there is variability in the final product from one patient to another. Partly to combat these and other drawbacks some industry groups are adding further steps – such as the additional cell sorting procedures to generate a defined-cell product – and these, while already showing evidence of better efficacy, of course add further to the time and cost.

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Confidence in CAR-T players was shaken at last year's ASH meeting when the University of Pennsylvania's Dr Stephen Schuster revealed a surprisingly high rate of production failures. In a study of Novartis's CTL019, in relapsed/refractory B-cell lymphomas, of 43 patients enrolled 13 could not be infused – six owing to production failure. Followers of a project that apparently is to be filed for regulatory approval next year could find this alarming.

Still, commercial manufacturing will be very different from basic methods being pursued by academia, and it is likely that in some patients with insufficient T cells manufacturing simply will not be attempted. Penn's Dr Stephan Grupp has suggested an alternative method, whereby cells are taken from a patient much earlier, though this too adds an extra level of logistical complexity. In the paediatric ALL study of CTL019 patients under three years old have had the highest manufacturing failure rate, and are being excluded from Novartis's multicentre trial, which the Swiss firm says is the largest clinical study of a CAR-T therapy. In ALL studies there has subsequently been better news on production failures, with Dr Jae Park saying the failure rate in his study of Juno's JCAR015 was under 5%.

How regulators might see things is another story. Paula Salmikangas, chair of the European regulator's CAT – the Committee for Advanced Therapies, which will oversee CAR-T approvals – says, "What worries me is the heavy, evolving nature of the science." This relates both to the addition and deletion of genetic material in cells, and manufacturing constraints and inter-patient comparability issues that might not become obvious until launch.

Her advice to applicants was to "start from the basics: a robust product, with robust manufacturing", and remember that if the production process changes then the cells will change too. She also stressed the need for early contact with the regulator, whether the applicant was a large or a small company. It is also important to remember how risky it is to rely on a single manufacturing plant; a recent internal manufacturing review of some NCI cell and gene therapy labs put a halt to all new patient dosing in the relevant studies (AACR – Mage-A3 double-whammy hits Kite, April 17, 2016).

Manufacturing efficiency feeds straight into the pricing debate. GlaxoSmithKline's head of cell therapy, Cedrik Britten, appeared to confirm critics' worst fears by suggesting at the Adopt Summit in London in March that the $178,000 per patient per year price tag of Amgen's Blincyto was a "range from which we can think about an increase" (Adopt Summit – Manufacturing is still the biggest hurdle for CAR-T, March 7, 2016). It is generally accepted that the commercial cost of a CAR-T therapy, including associated hospital costs, will be around $500,000 per procedure per patient; this is broadly in line with that of a stem cell transplant, so might be supportable for a "cure". However, since in most current haematology settings CAR-T is a procedure that acts as a bridge to transplant – merely clearing disease so that a patient can undergo stem cell transplantation – cost is a major consideration and could be hard to support.

Allogeneic CARs

One approach to overcome these issues might be to go down the allogeneic route, whereby an off-the shelf product is ready for use without the complexities of an autologous therapy, and it is well worth considering Cellectis here. This company has worked hard to transform itself from a failing crop protection player into a CAR-T company that has secured endorsement from Servier and none other than Pfizer. At the core of Cellectis's technology is a Talen nuclease-based gene editing technology that allows the T cells' native T-cell receptors to be knocked out to avoid the graft-versus-host disease that would be expected on administration of an allogeneic cell product into a non-matched patient.

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The CAR itself is a fairly standard second-generation construct, but Cellectis makes additional genetic alterations to its T cells, using the Talen technology. In addition to knocking out TCRα it can incorporate the RQR8 suicide gene, which might allow the cells to be ablated, by the infusion of Rituxan, in a patient suffering extreme toxicities. Also, the company is considering knocking out genes coding for CD52 or dCK to give the CAR-T cells resistance to lymphodepletion by fludarabine or alemtuzumab respectively. With future constructs that Cellectis intends to use against T-cell targets, for instance CD38 or CS1, it also wants to knock out these same antigens on the CAR-T cells to avoid cell fratricide.

Cellectis is still a preclinical player, but stole the limelight at the unveiling of abstracts for last year's ASH meeting. This detailed a case report of a single childhood ALL patient who had relapsed after Blincyto therapy and had successfully been treated with Cellectis's allogeneic UCART19 product by doctors at Great Ormond Street Hospital. The key was that the scientists were unable to generate autologous CAR-T cells from the child, so gave the off-the-shelf UCART19 as a last resort on a compassionate-use basis, achieving molecular remission.

Clearly, if this type of success can be repeated in a larger setting it could pose a significant threat to autologous CAR-Ts, whose application presents practical problems that will drive up their cost and might limit real-world use.

However, questions were raised at the unveiling of the ASH poster, which raised doubts about the actual benefit the patient got from UCART19 rather than Blincyto, and suggested that the patient had experienced some alloreactivity, suggesting incomplete T-cell receptor knockout. Other issues for Cellectis to tackle are overcoming the host clearing the (foreign) graft, and the possibility that increased hospital costs due to T-cell receptor knockout – rendering patients severely immunocompromised – could negate the cost advantage of allogeneic therapy.

In any case since the ASH abstract presentation Cellectis stock went into a protracted slump, largely as a result of Pfizer unexpectedly picking up rights to UCART19 from Servier, bypassing Cellectis, which effectively lost control over its lead asset in return for just $38.2m. It was not disclosed how much Pfizer paid Servier, however. There was also confusion about why Cellectis's chief executive, André Choulika, told an investor meeting just a day before the Pfizer deal that Servier could not exercise an option over UCART19 and thus sublicense it to a third party until after phase I studies (A bittersweet outcome for Cellectis, November 19, 2015).

A second case report of a paediatric patient successfully treated with UCART19 was unveiled by the Great Ormond Street Hospital group at the May 2016 meeting of the American Society of Gene and Cell Therapy. Clinical trials of UCART19, however, are not scheduled to begin until August 2016 – Clinicaltrials.gov lists two in a total of 212 patients. Pfizer already had a separate Cellectis alliance, covering 15 targets, which had been worth $80m up front.

Until recently Cellectis was the allogeneic CAR-T player to watch, but this is no longer the case, as demonstrated by the patent issued to Celyad last year covering TCR-deficient CAR-T cells – the same scientific approach that Cellectis uses. Thus, in a field where litigation is hardly a novel prospect, a patent battle between Cellectis and Celyad could be on the cards.

Novartis is also making early progress on a TCR-deficient universal CAR product, in its case through the use of Crispr/Cas. Of course, allogeneic cell products have their own issues, which Johnson & Johnson's scientific director Sicco Popma summarises as the "selection" problem. In other words, with allogeneic therapy the cells' origin is critical: "What is the cell source I can use for every patient? Who can provide it?" asked Mr Popma at this year's Adopt summit in London.

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Other manufacturing cost considerations

Juno has recently been championing the approach of Dr Stanley Riddell at Fred Hutchinson, focused on generating a defined-cell CAR-T product (see section above). It must at present be borne in mind that the additional complexity that this involves necessitates additional – costly – manufacturing steps. For instance, while a bulk T-cell product involves a single Clinimacs cell-sorting procedure, a defined 50:50 CD4+/CD8+ T-cell product like JCAR017 requires three. Each Clinimacs procedure costs $4,000-7,000, says Dr Alexey Bersenev, director of a cell therapy laboratory at Yale University.

Nevertheless, companies are working hard to bring down complexity and cost. Juno's Mr Levitsky, for instance, cites its acquisition of the German group Stage Cell Therapeutics (now known as Juno GmbH), whose technology includes "detachable reagents" that aim to automate serial selection of cells, meaning that it is no more expensive to sort for multiple cell types than it is for one. Moreover, Mr Levitsky admits that what some CAR-T researchers are doing in academic labs will never go forward as a commercial product.

It is also worth mentioning mRNA electroporation – a completely different method for getting genetic material coding for the CAR construct into a T cell. This is being championed by Maxcyte, a US company that recently completed a £10m ($14m) IPO on London's Aim. (Interview – Maxcyte's chance to turn CAR-T manufacturing on its head, May 25, 2016)

The vast majority of CAR-T groups use viral transduction – using either a lentiviral or gamma-retroviral vector to infect the T cells with the genetic material to express a CAR, which is then passed on as cells divide and expand. mRNA electroporation is based around the concept of transient CAR expression: an electrical field is used to make the cell membrane temporarily permeable, allowing mRNA coding for the construct to enter the cytoplasm. When the CAR is expressed it is transient – because there is no genetic integration the genetic material encoding the CAR is not passed on to progeny cells. One advantage that Maxcyte claims is that electroporation is much more efficient than viral transduction, resulting in some 80% of the T cells being transfected, versus under 50% for viral transduction.

This means that there is no need to expand the T cells after transfection, and the cells that are isolated after leukapheresis are the same ones that are put back, perhaps over multiple infusions. Thus the CAR-T product can be significantly more reproducible from patient to patient versus a virally transduced one, in which there is little control over which T cells population(s) expand. Most importantly, Maxcyte suggests that if its most advanced technology, dubbed Carma, works out it could cut the CAR-T manufacturing time down to under a day, implying a significant cost advantage for an autologous product.

Of course many issues remain to be resolved, and while transience of a CAR-T product could be advantageous in avoiding on-target/off-tumour effects the implied lack of persistence could simply mean that it is not efficacious enough. Maxcyte's earlier-generation mRNA electrophoresis technology is already being used by Dr Carl June's lab at University of Pennsylvania in four CAR-T projects in six separate clinical trials. Novartis appears to have no involvement in these.

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Studies of CAR-T projects transfected using mRNA electroporation

Source: EP Vantage, Clinicaltrials.gov

Project name	Antigen	Academic centre	Indication(s)	Enrolment	Trial ID
RNA CD123	CD123	University of Pennsylvania	AML	15	NCT02623582
19-28z+	CD19	Memorial Sloan Kettering	ALL	60	NCT01044069
RNA CART19	CD19	University of Pennsylvania	Hodgkin's lymphoma	16	NCT02277522
RNA CART19	CD19	University of Pennsylvania	Hodgkin's lymphoma	10	NCT02624258
CD20-specific T cells	CD20	Fred Hutchinson & NCI	lymphoma	12	NCT00621452
CD20-specific T cells	CD20	Fred Hutchinson & NCI	lymphoma	12	NCT00012207
RNA cMet CAR	c-Met	University of Pennsylvania	breast cancer	15	NCT01837602
RNA mesothelin SS1	mesothelin	University of Pennsylvania	pancreatic cancer	10	NCT01897415
RNA Meso-CIR T	mesothelin	University of Pennsylvania	mesothelioma	18	NCT01355965

Commercial threats and litigation

If CAR-T therapies suffer from a lack of amenable antigens on the one hand, they also face considerable competition in the narrow range of targets and indications where they have shown considerable promise. The anti-CD19 field, for instance, is extremely crowded, and there is unlikely to be sufficient room for more than the first few players to come to market.

Competitive threats from other technologies must also be borne in mind. One of the most obvious is Blincyto, Amgen's marketed anti-CD19 bispecific antibody; this is likely to be used before CAR-T therapy, meaning that if patients become resistant to it by way of antigen escape this would naturally make them ineligible for subsequent treatment with an anti-CD19 CAR. Meanwhile, checkpoint inhibitor antibodies like Bristol-Myers Squibb's Opdivo and Merck & Co's Keytruda are blazing a trail, and will likely raise the bar even further in solid tumours, even if their potential in haematology is significantly more limited. There is always potential to combine antibody immuno-oncology approaches with CAR-T, though the cost is a separate consideration.

Among other forms of adoptive cell therapy engineered T-cell receptors (TCRs) are often cited as a threat to CAR-T. Unlike CARs these can target internal antigens, presented on the surface of a cell, which represent a far greater range of targets than those cell-membrane proteins that can be targeted by CAR-T. However, TCRs are extremely complex since they additionally require matching with the haplotype of the recipient's human leukocyte antigen (HLA) type, and additional cell-sorting procedures to isolate the T-cell type that is capable of working with a particular type of receptor. Clinical progress here has been slow – the players include Adaptimmune, Juno, Kite and Bellicum – and commercially the threat is distant. Competition could also come from soluble TCRs such as those being developed by Immatics (Immatics answers the call from MD Anderson, August 26, 2015), or from antibody-coupled T-cell receptors being developed by Unum (Another endorsement as Unum goes beyond the CAR-T, June 9, 2015) or Purdue University/Endocyte.

The threat of litigation, arising from a relative lack of clarity over IP, is another consideration. Numerous academics have worked at different centres, and the resulting CAR-T work has in many cases been licensed to separate corporate entities, meaning that ownership could subsequently be disputed. For instance, Baylor College forms the basis of CAR-T work at Bellicum, Cellectis, Bluebird and Autolus. Indeed, a recent Cellectis paper in Nature covered a CAR-T on-switch that bore some resemblance to that being developed by Bellicum. And Celyad and Cellectis could separately clash over allogeneic CAR technology.

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That said, the biggest CAR-T-related patent dispute so far ended last year with a relatively benign settlement. This had pitted Juno versus Novartis, having started out as an action between St Jude Children's Hospital, the originator of a CAR-T construct later licensed by Juno, and University of Pennsylvania. At heart was whether Penn's Dr Carl June, a CAR-T pioneer, was capable of licensing biological material from St Jude, where he had worked, on to Novartis for commercial purposes.

Material transfer agreements between St Jude and Dr June suggest that he was limited to using the material up to phase I, and precluded transfer to third parties – implying that Dr June/Penn were not allowed to license the technology on to Novartis. In the event Novartis agreed to pay Juno $12.5m plus future milestones and "mid-single digit" royalties, thus ensuring that development of the groups' respective CAR-T projects could continue.

While a past result can in no way be a guide to the future, the settlement suggests that other unclear IP positions might be ironed out in a way that does not pose a crippling burden on either party. It is not inconceivable that cross-licensing arrangements will soon become the order of the day in CAR-T (A settling CAR-T development, April 7, 2015).

Kite last year filed an inter-parties review challenging, on the grounds of obviousness, the validity of a Memorial Sloan Kettering patent covering Juno's JCAR015.

Further problematic developments have quietly taken place, including for instance Sorrento Therapeutics, a company associated with Nantkwest, listing anti-CEA and anti-IL13R CAR-T assets in its pipeline; there is no indication where either of these was derived from. City of Hope is the group that has spearheaded development of anti-IL13R CAR-T cells, but this asset was licensed to Coronado Biosciences, a company that has since changed its name to Fortress Biotech/Mustang Bio. Sorrento did strike a licensing deal with City of Hope, but this related to monoclonal antibodies.

Another area that lacks clarity is Novartis's 2012 alliance with University of Pennsylvania. The original announcement stated that this deal covered the anti-CD19 CAR that was later designated CTL019, "as well as future CAR-based therapies developed through the collaboration". However, CAR-T work that was already in existence at the time presumably falls outside the deal, suggesting that rights to the mRNA electroporated projects mentioned above rest solely with Penn, as Maxcyte had been working on these with Dr Carl June, one of the leaders of the Penn work, since around 2008.

In January Dr June raised $10m for a separate private venture called Tmunity Therapeutics, to focus on the potential of T-cell treatments for a range of diseases, though for now all that is known is that this will focus on "T-cell receptor engineered T cells, regulatory T cells and universal engineered T-cell platforms".

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Where are we headed?

Early-stage assets

If it is clear that the CAR-T space suffers from a lack of antigen targets that are either expressed on tumour cells almost uniquely, or if not that offer a therapeutic window, various groups are not giving up.

CARs being tested preclinically offer an insight into where scientific and commercial thinking is headed (SITC preview – Macrogenics' time to shine, October 22, 2015). Studies are keenly awaited targeting antigens including CD123, CS1 and others, as well as CD19-directed constructs employing novel features and trials comparing different versions of the same basic CAR head to head. It will be especially interesting to watch the progress of Cellects's allogeneic approach against targets that the company has not yet licensed out.

A selection of preclinical CAR-T projects

Source: EP Vantage, company filings, academic presentations

Company	Academic source	Indication(s)	Project name	Antigen	Co-stim	Suicide gene	Note
none	NCI	B-cell malignancies	CD19/CD22 bispecific CAR	CD19 & CD22	4-1BB	none	bispecific CAR
Bellicum	Baylor College	B-cell malignancies	BPX-401	CD19	MyD88/CD40	iC9	–
Bellicum	Baylor College	various	HER2 CAR	Her2	MyD88/CD40	iC9	–
Cellectis	Weill Cornell	AML	UCART123	CD123	4-1BB	RQR8	allogeneic; TCRα & dCK knockout
Cellectis	MD Anderson	multiple myeloma, T-ALL	UCART38	CD38	4-1BB	RQR8	allogeneic; TCRα, CD38, dCK & PD-1 knockout
Cellectis	MD Anderson	multiple myeloma	UCARTCS1	CS1 (=CD319/ SLAMF7)	4-1BB	RQR8	allogeneic; TCRα, CS1 & PD-1 knockout
Cellectis	MD Anderson	ALL	UCART22	CD22	unknown	RQR8	allogeneic; TCRα & dCK knockout
Cellectis	unknown	multiple myeloma	UCART-BCMA	BCMA	unknown	unknown	allogeneic; TCRα knockout
Cellectis	unknown	pancreatic, NSCLC	UCART5T4	5T4	unknown	unknown	allogeneic; TCRα knockout
Cellectis	unknown	glioblastoma	UCART-EgfrVIII	EGFRvIII	unknown	unknown	allogeneic; TCRα knockout
Juno	none	B-cell malignancies	JCAR021	CD19	unknown	none	fully human binder
Juno	Fred Hutchinson Cancer Center	B-cell malignancies	Three CD19 CARs	CD19	4-1BBL vs CD40L	unknown	Trident trial; also compares vs IL-21 secreting CAR
Molmed	San Raffaele Hospital	various	CAR-CD44v6	CD44v6	unknown	unknown	–
Novartis	University of Pennsylvania	gynaecological cancers	B7-H4 CAR-T	B7-H4	4-1BB	none	–

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Geographical focus

At present the primary focus of these, as well as that of the initial wave of CAR-T players, is of course the US, the most important pharmaceutical market and the one most likely to support premium pricing. But other territories should not be ignored, though doubts as to companies' freedom to set prices must be several times greater in a regulated market like Europe than they are in the US. Nevertheless, both Novartis and Juno, through its partner Celgene, have made commitments to the EU.

Celgene recently exercised an option to buy ex-US/ex-China rights to Juno's CD19-targeting CARs. Rupert Vessey, Celgene's president of research and early development, recently told *EP Vantage*: "Juno is making significant steps and has invested heavily in turning this into a truly robust and scalable process, and that ultimately will be a big advantage for us and for them." Many Celgene executives have also stressed the need for greater collaboration between payers, patients and industry, to improve access to innovative treatments.

Celgene will soon have to make decisions about building its own Europe-focused manufacturing capacity, though what this will look like is far from clear. In the EU, where there are a number of regional regulators, it seems that it has yet to be determined whether a central manufacturing site will suffice (Interview – Despite the many unknowns Celgene banks on CAR-T take-off, April 18, 2016).

China

For Juno China forms a separate focus, as highlighted by an earlier alliance struck with Wuxi Apptec to form a local joint venture, JW Biotechnology. The aim is for JW to license CAR-T and engineered T-cell receptor candidates from Juno's pipeline for local development, backed by Wuxi's knowledge of the Chinese healthcare system and alliances with hospitals there (Juno looks east, April 8, 2016).

Given China's large population and appetite for novel science this makes perfect sense. Moreover, studies can be run relatively cheaply there and regulatory hurdles are low. The country is therefore likely to offer considerably cheaper therapeutic options than the US, making medical tourism a real possibility; given the limited financial outlay it pays for companies to have a hand in such a possibility, just in case. Still, Clinicaltrials.gov reveals the Chinese CAR-T market already to be competitive.

Clinical-stage CAR-T projects in China

Source: EP Vantage, Clinicaltrials.gov

Academic centre	Company	Project name	Antigen	Co-stim	Transfection*	ScFv	Suicide gene	Added feature(s)
Academy of Military Medical Sciences	none	3rd-gen CAR-T cells	CD19	CD28 & 4-1BB	lentivirus	unknown	none	–
Anhui Medical University	Sinobioway Cell Therapy	CD19 CAR	CD19	unknown	unknown	murine	none	–
Chinese PLA General Hospital	none	CD133-CAR	CD133	4-1BB	lentivirus	murine	none	–
Chinese PLA General Hospital	none	CART138 cells	CD138	4-1BB	lentivirus	murine	none	–
Chinese PLA General Hospital	Cellular Biomedicine Group	CBM-C19.1	CD19	4-1BB	lentivirus	murine	none	–

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Academic centre	Company	Project name	Antigen	Co-stim	Transfection*	ScFv	Suicide gene	Added feature(s)
Chinese PLA General Hospital	Cellular Biomedicine Group	CBM-C20.1	CD20	4-1BB	lentivirus	murine	none	–
Chinese PLA General Hospital	Cellular Biomedicine Group	CBM-C30.1	CD30	4-1BB	lentivirus	murine	none	–
Chinese PLA General Hospital	none	CART33 cells	CD33	4-1BB	probably lentivirus	murine	none	–
Chinese PLA General Hospital	Cellular Biomedicine Group	CMB-HER1.1	EGFR	4-1BB	lentivirus	murine	none	–
Chinese PLA General Hospital	none	CART-HER2	Her2	4-1BB	lentivirus	murine	none	–
Chinese PLA General Hospital	none	meso-CAR T	meso-thelin	4-1BB	probably lentivirus	murine	none	–
Fuda Cancer Hospital, Guangzhou	none	CD19-CAR	CD19	CD28	retrovirus	murine	none	–
Fuda Cancer Hospital, Guangzhou	none	CAR-T cell immunotherapy	EphA2	CD28	retrovirus	murine	none	–
Fuda Cancer Hospital, Guangzhou	none	CAR-T cell immunotherapy	GPC3	CD28	retrovirus	murine	none	–
Fuda Cancer Hospital, Guangzhou	none	HER-2-targeting CAR	Her2	CD28	retrovirus	unknown	none	–
Henan University of Traditional Chinese Medicine	none	CD19 CAR	CD19	CD28 vs 4-1BB	unknown	murine	none	–
Jichi Medical University	Takara Bio	CD19-CAR	CD19	CD28	retrovirus	murine	none	–
Jilin University	Beijing Doing Biomedical	CD19-CAR	CD19	unknown	unknown	unknown	unknown	–
Peking University	America Yuva Biomed	4SCAR19273	CD19	CD28, 4-1BB & CD27	lentivirus	unknown	iC9	–
Peking University	America Yuva Biomed	4SCAR30273	CD30	CD28, 4-1BB & CD27	lentivirus	unknown	iC9	–
RenJi Hospital	Carsgen	anti-EGFR CAR	EGFR	unknown	lentivirus	murine	none	–
RenJi Hospital	Carsgen	anti-GPC3 CAR	GPC3	CD28	lentivirus	murine	none	–
Second Military Medical University	none	nCAR19-T	CD19	unknown	retrovirus	murine	none	–
Shenzhen Second People's Hospital	none	CD19-CAR	CD19	CD28	lentivirus	unknown	undis-closed switch	–
Southwest Hospital, China	none	nCAR19-T	CD19	unknown	retrovirus	murine	none	–
Southwest Hospital, China	none	Anti-CD20-CAR	CD20	unknown	retrovirus	murine	none	–
Southwest Hospital, China	none	Anti-CEA-CAR	CEA	unknown	lentivirus	unknown	unknown	–
Tongji University School of Medicine	none	CD19-CAR-T	CD19	CD28	lentivirus	murine	none	–
Xinqiao Hospital of Chongqing	none	DSCAR01	CD19	unknown	retrovirus	murine	none	memory-enriched T cells

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Academic centre	Company	Project name	Antigen	Co-stim	Transfection*	ScFv	Suicide gene	Added feature(s)
Xinqiao Hospital of Chongqing	none	CD22-specific CAR	CD22	4-1BB	retrovirus	murine	none	–
Zhejiang University	America Yuva Biomed	4SCAR123	CD123	CD28, 4-1BB & CD27	lentivirus	unknown	iC9	–
Zhejiang University	Innovative Cellular Therapeutics	CD19 CAR	CD19	unknown	unknown	unknown	unknown	–
Zhi Yang	none	Anti-HER2 CAR	Her2	unknown	unknown	unknown	unknown	–
Zhujiang Hospital	America Yuva Biomed	4SCARGD2	GD2	CD28, 4-1BB & CD27	lentivirus	unknown	iC9	–
unknown	Sinobioway Cell Therapy	CD19 CAR	CD19	unknown	unknown	unknown	unknown	–
unknown	Beijing Doing Biomedical	CD19-CAR γδT	CD19	unknown	unknown	unknown	unknown	γδT cells
unknown	Shanghai Genechem	anti-CD19-CAR	CD19	4-1BB	lentivirus	murine	none	–
unknown	Sinobioway Cell Therapy	EPCAM CAR	EPCAM	unknown	unknown	unknown	unknown	–
unknown	Shanghai Genechem	TAI-GPC3-CART	GPC3	4-1BB	unknown	murine	none	transcatheter arterial infusion
unknown	Shanghai Genechem	TAI-meso-CART	meso-thelin	4-1BB	unknown	murine	none	transcatheter arterial infusion
unknown	Persongen Biomedicine	MUC1 CAR	MUC1	unknown	unknown	unknown	unknown	–

Note: *where retrovirus is stated this is likely a gamma-retrovirus, but since lentiviruses are a subtype of retroviruses it is possible that a lentivirus is being used.

The list includes not only the obvious targets – CD19-expressing leukaemias, for instance – but also solid tumours, as well as novel CAR constructs and enriched cell populations. Targeting CD22 for CD19-negative relapses, as Xinqiao Hospital of Chongqing is doing, mirrors Juno's own JCAR018.

Last year Cellular Biomedicine Group threw its hat into the ring through a RMB12m ($1.9m) deal to acquire CAR-T projects against CD19, CD20, CD30 and EGFR from the Chinese PLA General Hospital, which was already running studies in haematological as well as solid tumours. Earlier Cellular had paid $3.3m to buy Agreen Biotech, a Chinese company with technologies in T-cell receptor clonality analysis, and T central memory cell and dendritic cell preparation.

Since cell therapies are likely to rely on specific, regional hospitals and manufacturing plants capable of carrying out the complex procedures, aiming to become the leader with a network of authorised treatment centres throughout China makes sense. Cellular even boasts that it has a US FDA-compliant manufacturing plant in Shanghai (A move to roll up China's cell therapy market, February 11, 2015).

The Chinese group gained its Nasdaq listing through a reversal into EastBridge Investment Group in February 2013. However, the company shortly afterwards came under pressure from allegations over its disclosure, and in the past year its stock has lost about half its value.

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Carsgen Therapeutics is another local company, and claims to have a pipeline of seven CAR-T projects, including two – targeting GPC3 and EGFR – against hepatocellular cancer and glioblastoma respectively in clinical trials at Renji Hospital. Innovative Cellular Therapeutics claims to have achieved a 90% complete remission rate in 10 leukaemia patients treated at Zhejiang University, and to have carried out 23 clinical CAR-T studies.

There are likely to be many other projects and studies than specified in Clinicaltrials.gov, since Chinese companies have no obligation to list trials in this registry, and new commercial entities emerge frequently. For instance, in May Immune Therapeutics, a company traded on a US OTC exchange, said it had acquired CAR-T patents from Super-T Cell Cancer Company, a newly formed Chinese corporation.

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Conclusion

While much of the CAR-T work both in the West and in China is still early it should not go unnoticed how many studies have been initiated in the past year, and how much work has gone into trying to overcome many of the shortcomings and problems.

Indeed, it is important to remember that the CAR-T concept dates all the way back to the late 1980s; the many years of futile study of inappropriate targets and inefficient first-generation constructs should put the past three years' progress into perspective. An important element, of course, is that only since around 2013 – shortly after Novartis's buy-in to Penn's work gave the space an essential stimulus – have companies been able to secure large amounts of cash from private investors. Shortly before that, CAR-T research at Penn was seriously stagnating for lack of funding.

True, an awful lot still needs to be done, but the industry is well on the way to understanding toxicities, improving manufacturing, boosting cell persistence and CAR-T efficacy, and figuring out and overcoming the problem of relapses. What we still do not know is how receptive the fast-changing market will be to the realisation that much more time, money and effort is still needed, or indeed what will happen if one of the major players suffers a significant setback.

The fact that the regulatory path has yet to be tested means that FDA requirements can by and large only be guessed at. A noteworthy precedent was set when the world's first gene therapy, Gendicine, and first oncolytic virus, Oncorine, were approved in China, in 2003 and in 2005 respectively; the prospect of the first-ever commercially available CAR-T therapy being launched not in the West but in China is intriguing.

Many scientists active in this space see CAR-T within the broad context of adoptive T-cell therapies, starting with first-generation constructs, proceeding to the CD19 paradigm in haematology that could fulfil its potential over the next year or two. Beyond this lies CAR-T therapy for solid tumours, followed by T-cell therapy for infectious and autoimmune disease – a practically untouched area at present, but one that could see significant future research into the potential of genetically engineering T regulatory rather than effector T cells.

That, however, is still a long way away. Before these heights can be scaled the industry needs to generate positive pivotal data in the first indications, and prove that CAR-T really can be commercially viable.

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