ORIGINAL RESEARCH ARTICLE

A Phase 1 Study of Intravenous EGFR-ErbituxEDVsMIT in Children with Solid or CNS Tumours Expressing Epidermal Growth Factor Receptor

Louise Evans[1] · Rick Walker[2,3] · Jennifer MacDiarmid[4] · Himanshu Brahmbhatt[4] · Antoinette Anazodo[5,6] · Geoffrey McCowage[7] · Andrew J. Gifford[6,8,9] · Maria Kavallaris[10,6,9] · Toby Trahair[5,6] · David S. Ziegler[5,6,9]

Abstract

Background Recurrent or refractory solid and central nervous system (CNS) tumours in paediatric patients have limited treatment options and carry a poor prognosis. The EnGeneIC Dream Vector (EDV) is a novel nanocell designed to deliver cytotoxic medication directly to the tumour. The epidermal growth factor receptor is expressed in several CNS and solid tumours and is the target for bispecific antibodies attached to the EDV.

Objective To assess the safety and tolerability of EGFR-Erbitux receptor EnGeneIC Dream Vector with mitoxantrone ($^EEDVs_{Mit}$) in children with recurrent / refractory solid or CNS tumours expressing EGFR.

Patients and methods Patients aged 2–21 years with relapsed or refractory CNS and solid tumours, or radiologically diagnosed diffuse intrinsic pontine glioma (DIPG), were treated in this phase I open-label study of single agent $^EEDVs_{Mit}$. Thirty-seven patients' tumours were screened for EGFR expression. $^EEDVs_{Mit}$ was administered twice weekly in the first cycle and weekly thereafter. Standard dose escalation with a rolling 6 design was employed. Dosing commenced at 5×10^8 $^EEDVs_{Mit}$ per dose and escalated to 5×10^9 $^EEDVs_{Mit}$ per dose.

Results EGFR expression was detected in 12 (32%) of the paediatric tumours tested. Nine patients were enrolled and treated on the trial, including three patients with diffuse midline glioma. Overall, $^EEDVs_{Mit}$ was well tolerated, with no dose-limiting toxicities observed. The most common drug-related adverse events were grade 1–2 fever, nausea and vomiting, rash, lymphopaenia, and mildly deranged liver function tests. All patients had disease progression, including one patient who achieved a mixed response as the best response.

Conclusions EGFR-Erbitux receptor targeted EnGeneIC Dream Vector with mitoxantrone can be safely delivered in paediatric patients aged 2–21 years with solid or CNS tumours harbouring EGFR expression. The discovery of EGFR expression in a high proportion of paediatric gliomas means that EGFR may be useful as a target for other treatment strategies. Targeted therapeutic-loaded EDVs may be worth exploring further for their role in stimulating an anti-tumour immune response.

ClinicalTrials.gov Identifier NCT02687386.

Key Points

A novel therapy using a nanocell to deliver chemotherapy directly to the tumour is well tolerated in paediatric patients.

In this study one in three patients with relapsed or refractory brain tumour or solid tumours showed expression of the epidermal growth factor receptor, which can potentially be used as a therapeutic target.

Extended author information available on the last page of the article

1 Introduction

Children with relapsed and refractory solid or central nervous system (CNS) tumours have limited effective treatment options and carry a poor prognosis. Traditional chemotherapeutic agents commonly used in this setting are constrained by systemic toxicity when delivered at the doses required for therapeutic benefit [1, 2]. An alternative approach to reduce toxicity to normal cells is to encapsulate the chemotherapeutic drug in a nanovector and deliver the drug intracellularly directly to tumour cells. Such "active targeting" requires a receptor at the tumour cell surface [2].

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The EnGeneIC Dream Vector (EDV) is a bacterial minicell: an anucleate, non-living 400 nm diameter nanocell produced through inactivation of the genes that control normal bacterial cell division [3]. The EDV packages a chemotherapeutic agent and carries a bispecific antibody targeting specific cancer cells [2, 4]. The EDVs carry a payload of up to 1 million drug molecules, which allows a high concentration of chemotherapy to be delivered and thereby potentially increase the potency of the antitumour effects [2]. The EDVs can be targeted to receptors on cancer cells, such as EGFR, using bispecific antibodies. Following intravenous administration, the minicells extravasate into the tumour microenvironment due to leaky vasculature associated with solid tumours. The leaky vasculature results in an enhanced permeation-retention effect, which promotes accumulation of nanoparticles in tumour tissue compared to normal tissues [2, 5].

It has been demonstrated in different tumour xenograft studies that the minicells extravasate into the tumour environment via the leaky vasculature and do not extravasate into any of the other normal tissues [5]. This is likely because the minicells are 400 nm in diameter and the largest fenestrations found associated with normal vasculature are 100 nm or less [4]. EDV studies using microRNA (miRNA) also show that miRNA is delivered to tumour tissue with anti-tumoral effect in mouse models with medullary thyroid carcinoma, mesothelioma and glioblastoma [6–8]. Rapid accumulation of doxorubicin-containing EDVs, $^{E}EDVs_{Dox}$, in the core of brain tumours was demonstrated in preclinical studies including 17 dogs with glioblastoma, using 123-iodine labelled minicells [5]. The minicells are macropinocytosed and following EDV breakdown in lysosomes, the cytotoxic drug is released into the tumour cell [2].

The EDV selectively targets the cancer cell via the bispecific antibody; one arm has specificity to the polysaccharide of the minicell and the other to the EGFR. EGFR (also known as ErbB-1) is a transmembrane tyrosine kinase and is a product of the *c-erbB1* proto-oncogene [9]. EGFR is important in cell proliferation, differentiation, invasion, survival and angiogenesis [10]. EGFR is expressed in several CNS and solid tumours in adults and is associated with a poor prognosis [1, 11, 12]. EGFR expression may therefore be over-represented in patients with recurrent/refractory solid or CNS tumours and is an attractive target. The EGFR-Erbitux receptor-targeted EDV does not inhibit EGFR function, and thus requires EGFR expression for cellular internalisation, rather than intracellular signalling.

A number of clinical trials using EDVs in small and large animals have shown safety, tolerability and clinical response. Preclinical studies of EDVs packaged with mitoxantrone in murine xenograft models with colon cancer resulted in tumour stabilisation or regression [2]. EGFR-antibody linked EDVs have been shown to enter and kill paediatric neuroblastoma cells in vitro and in vivo in murine models [13, 14]. Tumour regression was shown in two dogs with advanced stage Hodgkin's lymphoma [2]. EGFR-targeted minicells loaded with doxorubicin were administered to 17 dogs with spontaneously occurring late-stage brain cancer and clinical activity was observed, including two dogs with complete response [5].

Clinical trials have been conducted in adult patients with solid and CNS tumours. Whittle et al. describe the use of doxorubicin-containing EDVs in adult patients with recurrent glioblastoma [15]. Overall, the treatment was well tolerated, with no dose-limiting toxicity and no withdrawals from the study due to adverse events [15]. Solomon et al. described 22 adult patients who completed at least one cycle of EGFR-targeted, paclitaxel-loaded EDVs. The treatment was well tolerated and ten patients (45%; n = 22) achieved stable disease as their best response [1].

The EDV is loaded with a cytotoxic chemotherapy agent. In preclinical studies and clinical trials in adults the EDVs were loaded with the anthracycline doxorubicin. Mitoxantrone, also an anthracycline, has an established safety profile in the paediatric population and is active across a broad range of malignancies. We conducted a high throughput screen in paediatric high-grade gliomas that identified mitoxantrone as a potent anti-tumour agent in vitro. Mitoxantrone is not commonly used as a first-line therapy for paediatric solid or CNS tumours, and therefore acquired tumour resistance arising from previous exposure was considered less likely compared with doxorubicin. Therefore, mitoxantrone was selected as the anthracycline for use in this trial.

This paper describes the results of a phase I study of the use of mitoxantrone-containing EDVs targeting EGFR in paediatric patients with EGFR expressing relapsed/refractory solid or CNS tumours. We describe the proportion of patients with EGFR expression, safety and tolerability of the treatment, and preliminarily define the anti-tumour activity of mitoxantrone-containing EDVs.

2 Methods

2.1 Study Design

This was an open-label, sequential dose exploration study of single-agent EGFR-Erbitux receptor EnGeneIC Dream Vector with mitoxantrone ($^{E}EDVs_{Mit}$) administered by

intravenous infusion in children with recurrent/refractory solid or CNS tumours with EGFR expression or diffuse intrinsic pontine glioma (DIPG). Patients aged 2–21 years with recurrent/refractory solid/CNS tumours were enrolled, following a minimum of first-line therapy. Evidence of expression of EGFR in tumour biopsies, as assessed by immunohistochemistry (IHC), was required except in patients with DIPG who did not have a prior biopsy. Staining was reviewed by a pathologist and tumours with more than 20% of tumour cells demonstrating membranous (partial or complete) staining were scored as positive. Patients with a radiologic diagnosis of DIPG were eligible without prior biopsy. Patients were eligible for treatment only if they had fully recovered from the acute toxic effects of all prior therapies: at least 3 weeks post myelosuppressive chemotherapy; 7 days post biologic agents; 6 weeks post immunotherapy, MIBG therapy and radiotherapy; and 12 weeks post bone marrow transplant. All patients were screened for antibodies to *Salmonella typhi* prior to enrolment, with a positive result leading to exclusion from the study as the minicell is derived from this bacterial cell wall. Enrolment in the study required informed patient consent and the study was approved by the Sydney Children's Hospital Network Human Research Ethics Committee (HREC). ClinicalTrials.gov Identifier: NCT02687386

The dose-exploration study used a standard dose escalation with a rolling 6 design. Dosing was guided by prior adult recurrent glioma trials; dosing commenced at one log scale below the maximum tolerated dose (MTD) in adults [15]. Dose level 0 was administered at 5×10^8 EDV per dose, dose level 1 was administered at 2.5×10^9, and dose level 2 tested the maximum dose level of 5×10^9 EDV per dose. At each dose level, the first four doses administered to each patient were administered at 10% of the target dose, before escalation to the target dose for all subsequent doses in the absence of any dose-limiting toxicities.

2.2 Treatment

Participants received $^{E}EDVs_{Mit}$ by intravenous injection twice weekly as a 20-min infusion beginning at study day 1 for the first 28-day cycle, then weekly for subsequent cycles. $^{E}EDVs_{Mit}$ contains approximately 600 µg of mitoxantrone and 5 ± 0.5 µg of anti-human EGFR (Erbitux sequence) bispecific antibody per 1×10^9 EDVs. All patients received premedication with dexamethasone, antihistamine (promethazine or loratadine) and paracetamol 30–60 min prior to the infusion. Treatment was administered until dose-limiting toxicity (DLT), disease progression, revocation of consent or cessation of trial medication production.

2.3 Toxicity Evaluation

Adverse events were graded by severity in line with the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The relationship of the adverse event to the treatment was determined by the Investigator. DLTs were defined as clinically significant grade 3 or 4 non-haematologic toxicity, except fatigue. Nausea and vomiting were considered a DLT only if persisting longer than 7 days despite medical management. Haematologic DLTs were defined as grade 4 neutropeania, grade 3 thrombocytopaenia with bleeding or grade 4 thrombocytopaenia.

2.4 Response Evaluation

Patients underwent magnetic resonance imaging (MRI) or computed tomography (CT) assessment of their tumours at enrolment, after the second cycle of EDV therapy, and every second cycle thereafter. The modified response evaluation criteria in solid tumours (RECIST) criteria were used to assess response to treatment [16]. Blood samples were taken pre and post each cycle and peripheral blood mononuclear cells (PBMCs) were isolated. Changes in natural killer cell subsets (NK) and CD8+T cells were examined by Beckman Gallios flow cytometer using CD56, CD56dim CD16+ and CD56dim CD16- as markers for NK cells and CD45, CD3+ and CD8+ for cytotoxic T-cells.

2.5 Immunogenicity Evaluation

Serum samples pre-dose and 3 h post-dose were analysed for levels of the inflammatory cytokines tumour necrosis factor alpha (TNFα) and interleukin 6 (IL-6) following the first, second and fifth doses in cycle 1 and after the first dose only in subsequent cycles.

2.6 Statistics

Descriptive statistics were used to describe the proportion of patients with EGFR positivity and grading of adverse effects. The effect of dose level on inflammatory cytokine levels was assessed using a random-effects Tobit regression model. Event-free survival as a function of time since enrolment was measured using the Kaplan-Meier method.

Table 1 EGFR status of tumours evaluated for study enrolment

	Total tested for EGFR status	Total EGFR positive (%)
High grade glioma/glioblastoma	9	7 (78%)
Diffuse midline glioma	10	4 (40%)
Medulloblastoma	6	0 (0%)
Other/non-CNS solid tumours	12	1 (8%)
Total	37	12 (32%)

EGFR epidermal growth factor, *CNS* central nervous system

3 Results

3.1 EGFR Positivity

Patients were enrolled between August 2016 and June 2018. The EGFR status of 37 patient tumours was evaluated (Table 1). EGFR testing was positive by IHC in 12 of 37 patients (32%). Most patients with EGFR-positive tumours had either high-grade gliomas or diffuse midline gliomas (DMG). Seven out of nine patients diagnosed with either high-grade glioma or glioblastoma were positive for EGFR. All patients with DMG had biopsies except for DIPG patients who were biopsied at the clinician's discretion. Four out of ten biopsied DMG patients had positive staining but were not enrolled in the trial and did not receive treatment with $^{E}EDVs_{Mit}$. Notably no medulloblastoma patients were EGFR positive. Eleven patients with an extra-cranial solid tumour were tested and only one tumour was found to have EGFR expression.

3.2 Baseline Characteristics

Nine patients were enrolled in the therapeutic study and received treatment with $^{E}EDVs_{Mit}$. Baseline characteristics are outlined in Tables 2 and 3. Patients were aged 5–18 years, with a median age of 9 years. Seven female and two male patients were enrolled. Five patients had a diagnosis of high-grade glioma/glioblastoma, three patients had a radiologic diagnosis of DIPG without prior biopsy, and one patient had a non-CNS solid tumour (sclerosing epithelioid fibrosarcoma). All patients were heavily pretreated and had disease progression after previous therapy

Table 2 Baseline characteristics of treated patients

Sex	Male		2 (22%)
	Female		7 (78%)
Age	Median		9 years
	Range		5–18 years
Tumour type	CNS	High grade Glioma/glioblastoma	5 (55%)
		Diffuse midline glioma	3 (33%)
	Solid tumour	1 (11%)	
Previous Therapy[a]	Surgery		4 (44%)
	Radiotherapy		8 (88%)
	Chemotherapy		2 (22%)
	Other		2 (22%)

CNS central nervous system

[a]Patients may have received more than one previous therapy

Table 3 Previous therapy per participant

Age, y	Sex	Tumour type	Previous therapy
5	Female	High-grade glioma	Surgery + chemotherapy + radiotherapy
15	Female	Glioblastoma	Surgery + chemotherapy + radiotherapy
12	Female	Glioblastoma	Surgery + radiotherapy + immunotherapy
9	Female	High-grade glioma	Radiotherapy
5	Male	Diffuse intrinsic pontine glioma	Radiotherapy
6	Female	Diffuse intrinsic pontine glioma	Radiotherapy
7	Female	Glioblastoma	Radiotherapy
14	Male	Diffuse intrinsic pontine glioma	Radiotherapy + hyperthermia + vitamin infusions
18	Female	Sclerosing epithelioid fibrosarcoma	Surgery

with either surgery, radiotherapy, chemotherapy, immuno-therapy or a combination of these treatment modalities. The time between surgery and EDV delivery was 14–76 weeks (median 28 weeks) and time from radiotherapy to EDV delivery was 9–41 weeks (median 17 weeks). One patient had chemotherapy more than 12 months prior to EDV ther-apy, followed by radiotherapy 3 months prior to enrolment, and one patient had chemotherapy at least 10 weeks prior to EDV therapy. Two patients had other therapy at least 11 weeks prior to EDV therapy, including hyperthermia, vita-min infusions and immunotherapy.

3.3 Treatment Administered

Patients received treatment at three dose levels, as summa-rised in Table 4. Three patients received treatment at dose level 0 (5×10^8), three at dose level 1 (2.5×10^9) and three at dose level 2 (5×10^9). The minimum number of doses administered was eight doses and the maximum was 28 doses. Three patients received more than 12 doses.

3.4 Adverse Effects

A total of 171 adverse events were reported throughout the study period, of which 72 were deemed unrelated to the study drug. No DLTs were recorded. A total of 99 adverse events were deemed at least potentially related to the study drug (Table 5). The most common included fever, nausea, vomiting and rash. Biochemical abnormalities included lym-phopaenia and mildly elevated transaminases.

Most adverse events were classed as grade 1 toxicity ($106/170 = 62\%$) and only two events reached grade 4 tox-icity (both lymphopenia, which was not defined as a DLT). One patient had grade 3 nausea and vomiting, which lasted less than 7 days so was not considered a DLT. Most adverse events were self-limiting, had no long-term sequelae, and led to no change in the treatment regimen. Fevers were all

classified as Grade 1, most occurred immediately post infu-sion of the study drug, were self-limiting and not associated with systemic toxicity. Treatment with the study drug was ceased in one patient due to vomiting and headache in the setting of hydrocephalus, and this was ultimately deemed unrelated to the study drug. One patient had a temporary interruption of treatment due to facial nerve palsy, deemed unrelated to the study drug.

3.5 Immunogenicity

Antibodies to *Salmonella typhi* (anti-LPS) at screening were negative in all patients screened for the study.

Predose TNFα was undetectable in all patients treated at all dose levels. There was an increase in TNFα at the 3 h post-dose timepoint at all dose levels (Fig. 1). The mean post dose TNFα level was 22.7 pg/mL in the patients treated at dose level 0, 71.7 pg/mL in those treated at dose level 1, and 84.5 pg/mL in those treated at dose level 2. There was a statistically significant difference between the mean TNFα at dose level 0 compared with dose level 1 ($p < 0.02$) and dose level 0 compared with dose level 2 ($p < 0.001$). The median post-treatment TNFα at dose level 1 was approximately 5.6 times higher [95% confidence interval (CI) 1.31–23.72] than that of dose level 0. The median post-treatment TNFα at dose level 2 was approximately 16.6 times higher (95% CI 3.81–72.25) than that of dose level 0. This suggests increased immunogenicity resulting from study treatment at higher dose levels.

There was no detectable pre-dose IL-6 in all patients except one. There was an increase in 3-h post dose IL-6 levels at all dose levels (Fig. 1). The mean 3-h post-treatment level of IL-6 was 3479.8 at dose level 1, 6349.4 at dose level 2, and 8453.9 at dose level 3. There is no evidence for an overall difference in 3-h post-treatment IL-6 levels based on dosing level of EDV ($p = 0.372$). Two DMG patients had increases in naïve CD8+ T cells and cytotoxic effector T

Table 4 Summary of dose levels administered

Dose level cohort		Participant	Number of doses received	Dose limiting toxicity	Best response	Survival (months)
0	5×10^8 (first 4 doses at 5×10^7)	0005	10	Nil	PD	5
		0016	14	Nil	PD	8
		0017	18	Nil	PD	5
1	2.5×10^9 (first 4 doses at 2.5×10^8)	0001	10	Nil	PD	3
		0014	9	Nil	PD	3
		0020	10	Nil	PD	22
2	5×10^9 (first 4 doses at 5×10^8)	0022	28	Nil	PD	9
		0032	8	Nil	PD	2
		0037	9	Nil	PD	3

PD progressive disease

Table 5 Adverse events related to study drug

	Total number of episodes	Grade 1	Grade 2	Grade 3	Grade 4
Clinical					
Fever	13	13	0	0	0
Tachycardia	5	1	4	0	0
Headache	6	4	2	0	0
Fatigue	4	0	3	1	0
Nausea and vomiting	8	4	3	0	0
Neurologic	3	3	0	0	0
Rash	7	7	0	0	0
Hypotension	1	1	0	0	0
Biochemical					
Leukopenia	5	4	1	0	0
Lymphopenia	16	7	0	7	2
Anaemia	3	2	1	0	0
Thrombocytopenia	2	2	0	0	0
Neutropenia	1	1	0	0	0
LFT derangement	17	16	1	0	0
Other, e.g., electrolyte disturbance, raised CRP	8	8	0	0	0
Total	99	73	15	9	2

LFT liver function test, *CRP* C-reactive protein

cells following dosing with $^{E}EDVs_{Mit}$, as well as increases in a unique subset of NK cells, $CD56^{dim}$ $CD16^{-}$ (Fig. 2).

3.6 Outcomes

3.6.1 Tumour Response

Imaging-based tumour response data was available for five patients. All patients had progressive disease. One patient had stable disease following cycle 2 of treatment; however, they had no further imaging and had clinical disease progression. Four patients had progressive disease following cycle 2 of EDV-based therapy. One patient had progressive disease after cycle 2; however, this patient was clinically well and believed to be benefiting from treatment, so the decision was made to continue treatment. Following cycle 4, this patient had a mixed response as some lesions decreased in size and new lesions appeared. Four patients did not have repeat imaging after the baseline MRI but had clinical evidence of disease progression by the end of the first cycle.

3.6.2 Mortality

All nine treated patients died within the follow-up period, as shown in Fig. 3. One patient with DIPG had a 22-month survival. She received one cycle of study drug but was removed from study due to parental concerns regarding travel constraints. The cause of death for all patients was

disease progression. One patient had pneumonia recorded as a secondary cause of death. No deaths were attributed to the study medication.

4 Discussion

Treatment options for patients with relapsed/refractory solid and CNS tumours are extremely limited and these patients unfortunately have a very poor prognosis. There is a clear need to identify novel therapeutic treatment options for this high-risk patient cohort.

This phase I dose-escalation trial was the first study of bi-specific antibody-targeted cytotoxic drug-packaged minicells in a paediatric patient cohort. Our findings showed that treatment with EGFR-targeted minicells loaded with mitoxantrone is safe and well tolerated in paediatric patients. The highest planned dose level was reached, and three patients were treated at this level; however, the trial was ceased before a recommended phase 2 dose could be definitively determined due to discontinuation of the study drug. However, there were no DLTs reported, even at the higher dose levels. The most common adverse events were fever, nausea and vomiting, rash, lymphopenia, and mildly deranged liver function tests. The majority of adverse events were mild and self-limiting, and there were no treatment-related deaths.

The administration of bacterial minicells packaged with chemotherapeutic agents resulted in a mild transient rise in
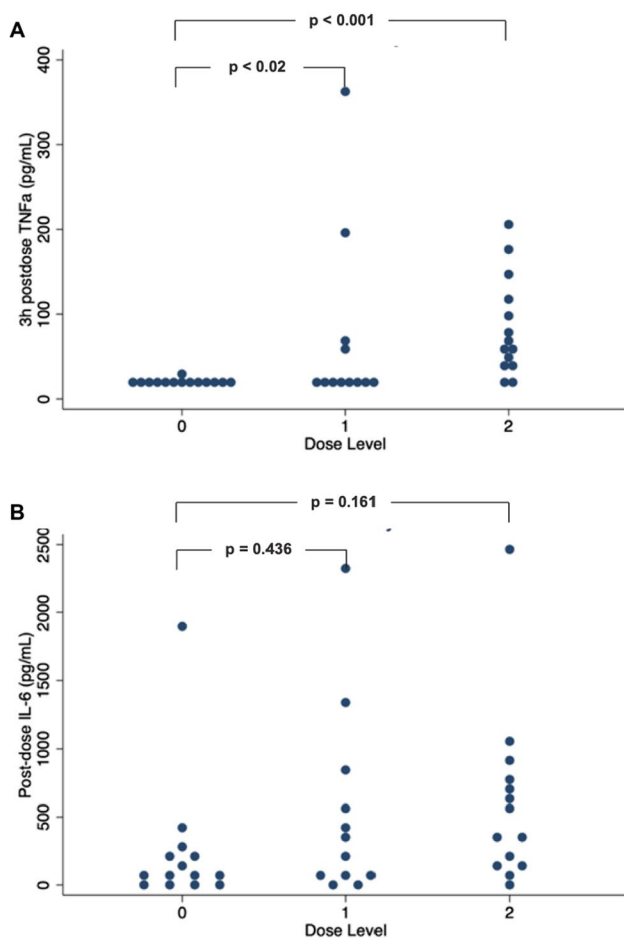
Fig. 1 Treatment effect on TNFα and IL-6. TNF alpha levels (**A**) and IL6 levels (**B**) were measured 3 h post administration of the study drug for patients treated at each dose level. TNFα levels significantly increased with increasing dose. The increase in IL-6 did not reach statistical significance. *p* values calculated by random-effects Tobit regression model

inflammatory cytokines. A more robust TNFα response than IL-6 response was noted. The inflammatory response may be due to the bacterial origin of the minicells [15]. Bacterial cell wall components such as lipopolysaccharides (found in the minicell outer membrane) are known to bind to receptors on the macrophage and initiate a signal transduction cascade, resulting in TNFα transcription [15]. However, TNFα production is tightly controlled to avoid pathological consequences. The anti-inflammatory cytokine IL-10 inhibits TNFα mRNA expression through activation of the STAT3 transcription factor pathway and subsequent expression of STAT3-dependent gene products [17]. In previous clinical trials, initial doses of minicells resulted in a serum spike of TNFα within physiological levels without any adverse effects, 3 h post minicell dose. At the same time points, a spike in IL-10 was observed, consistent with earlier observations that IL-10 was a moderator of TNFα. Interestingly,

as subsequent minicell doses were administered, the TNFα spike gradually tapered off by doses five to ten, resulting in immune tolerance to the minicell associated LPS [18]. Immune tolerance was not observed in our study, although it is possible some patients did not receive enough treatment doses to develop immune tolerance. The induction of an immune response raises the hypothesis that EDV could be combined with immunotherapies to enhance their activity. Also, it has been shown in pre-clinical animal studies and in humans that the targeted EDV carrying a payload itself induces an innate and adaptive anti-tumour response [14]. While the cohort treated was small, immune cell analysis showed that after two cycles, two DMG patients had an increase in the CD56^{dim} CD16^{-} subtype of NK cells, which are a unique non-cytolytic subset that have shown correlation with better patient outcome in some tumours [19]. Naïve CD8+ T cells as well as those that had differentiated into cytotoxic effector T cells to kill cancer cells were also elevated in these patients. This response was not sustained, possibly due to an insufficient number of EDV doses administered or an insufficient dose to allow significant numbers of cytotoxic T cells to accumulate in the tumour microenvironment [14]. Future studies may benefit from using iRECIST rather than RECIST criteria to assess treatment response since targeted and drug-loaded EDVs behave as a cyto-immunotherapeutic [14].

The EGFR receptor has been identified as a viable target for targeted therapy, although its role as a target in paediatric solid and CNS tumours remains to be defined. Unlike in adult high grade glioma (HGG), EGFR is only rarely mutated or amplified in paediatric HGG; however, the proportion of CNS and solid tumours expressing EGFR as a target for conjugated antibodies has not previously been well described. In this study we observed a difference in the proportion of patients with EGFR tumours based on tumour type. EGFR positivity by IHC was observed in a high proportion of patients with a high-grade glioma or glioblastoma. The results of this study may be used in the development of further treatment options using EGFR as a target for treatment with therapies such as EDV or bispecific antibodies that rely on EGFR expression rather than mutation or amplification. Alternative EDVs are in development with mitoxantrone replaced with different payloads, which will address multi-drug resistance.

The best anti-tumour response observed in this study was a mixed response to treatment. The small numbers of patients treated at the highest dose-level may impact these findings.

This study has several limitations, particularly due to the small cohort enrolled and treated with the bacterial minicells and the early cessation of the trial. An EDV packed with a different chemotherapeutic payload may improve treatment response; however, this was not tested in this phase

Fig. 2 Naïve and effector CD8+ T cells and NK cell subtype CD56DimCD16-. The percentage of cytotoxic effector T cells in CD8+ T cells, percentage naïve T cells and CD8+ T cells and percentage CD56dim CD16- in NK cells was tested in two patients (**A**, **B**). Timepoints depicted are: 1. Screening; 2. Completion of Cycle 1; 3. Completion of Cycle 3
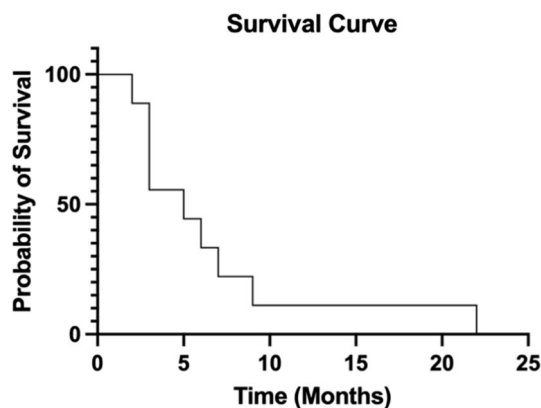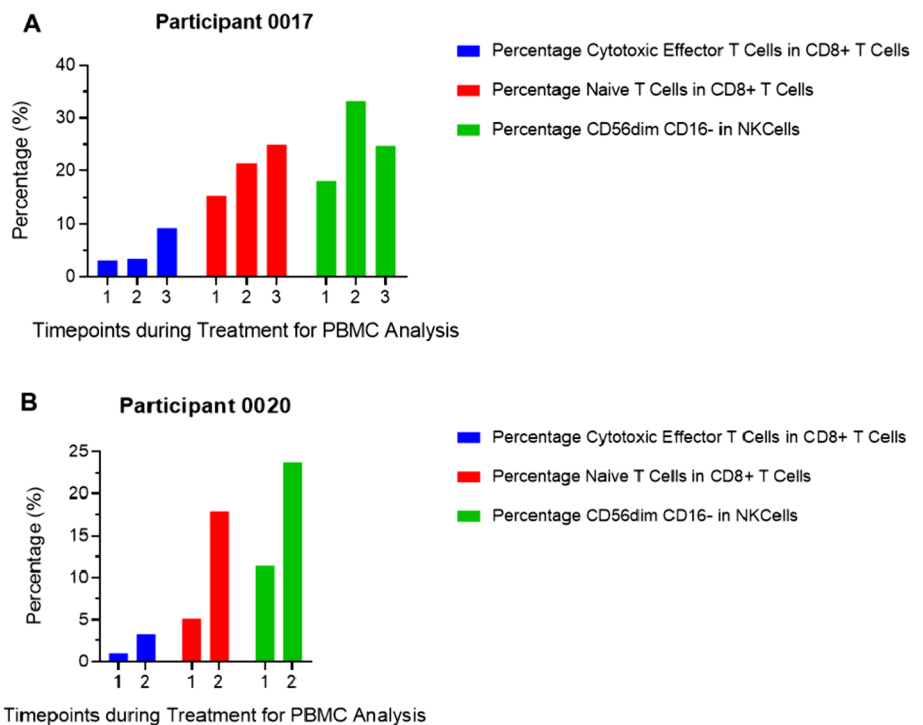

Fig. 3 Patient survival. Kaplan–Meier overall survival curve of patients who were treated with study drug

I trial and could be the subject of future research. Three patients with DIPG were enrolled without biopsy confirmation of EGFR positivity. It is possible this cohort did not have EGFR positivity, confounding efficacy assessment in these patients.

5 Conclusion

This study demonstrates that $^{E}EDVs_{Mit}$ (EGFR-Erbitux receptor targeted EnGeneIC Dream Vector with mitoxantrone) can safely be delivered in paediatric patients aged 2–21 years with relapsed or refractory solid or CNS tumours harbouring EGFR expression. The discovery of EGFR expression in a high proportion of paediatric gliomas means that EGFR may be useful as a target for EDVs carrying payloads to overcome drug resistance or for other targeted treatment strategies.

Declarations

Consent for publication Consent to enrolment on study included consent for publication.

Availability of data and materials The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Author contributions David S. Ziegler, Jennifer MacDiarmid, Himanshu Brahmbhatt, Rick Walker, Antoinette Anazodo, Geoffrey McCowage, Andrew J. Gifford, Maria Kavallaris and Toby Trahair contributed to the study conception and design. Material preparation, data collection and analysis were performed by Louise Evans and David S. Ziegler. The first draft of the manuscript was written by Louise Evans and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Authors and Affiliations

Louise Evans[1] · Rick Walker[2,3] · Jennifer MacDiarmid[4] · Himanshu Brahmbhatt[4] · Antoinette Anazodo[5,6] · Geoffrey McCowage[7] · Andrew J. Gifford[6,8,9] · Maria Kavallaris[10,6,9] · Toby Trahair[5,6] · David S. Ziegler[5,6,9]

✉ David S. Ziegler
d.ziegler@unsw.edu.au

1 Michael Rice Centre for Haematology and Oncology, Women's and Children's Hospital, North Adelaide, SA 5006, Australia

2 Queensland Children's Hospital, South Brisbane, QL 4101, Australia

3 School of Medicine, The University of Queensland, Herston, QL 4006, Australia

4 EnGeneIC, Sydney, NSW 2066, Australia

5 Kids Cancer Centre, Sydney Children's Hospital, Randwick, NSW 2031, Australia

6 School of Clinical Medicine, Faculty of Medicine and Health, University of New South Wales, Sydney, NSW 2052, Australia

7 The Children's Hospital, Westmead, NSW 2145, Australia

8 Anatomical Pathology, NSW Heath Pathology, Prince of Wales Hospital, Randwick, NSW 2031, Australia

9 Children's Cancer Institute, Lowy Cancer Research Centre, University of New South Wales, Sydney, NSW 2052, Australia

10 Australian Centre for Nanomedicine, University of New South Wales, Sydney, NSW 2052, Australia