Overcoming target epitope masking resistance that can occur on low-antigen-expresser AML blasts after IL-1RAP chimeric antigen receptor T cell therapy using the inducible caspase 9 suicide gene safety switch

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Abstract
Although chimeric antigen receptor CAR) T cell immunotherapies are an undeniable and unequivocal success, knowledge obtained from the monitoring of the first clinical trials targeting the CD19 antigen in B malignancies, either refractory/relapsed acute lymphoid leukemia (ALL) or lymphomas, contributed to the identification of tumor cell escape in about 30–50% of B-ALL. Resistance occurred due to loss of surface expression of the antigen (rCD19−) or to the early disappearance or inactivation of CAR T cells (rCD19+). In a recently reported clinical case, rCD19− relapse resulted from masking of the antigen by the CAR at the surface of B-ALL leukemia cells following the unexpected viral transduction of a leukemic cell present in the cytopheresis sample. The objective of this work was to reproduce this epitope-masking resistance model, in the context of acute myeloid leukemia (AML), based on our immunotherapeutic CAR T cell model targeting the accessory protein of the interleukin-1 receptor (IL-1RAP) expressed by leukemic stem cells. As AML primary blasts express different levels of IL-1RAP, we modeled transduction of different AML tumor cell lines screened for density of antigenic sites with our lentiviral vectors carrying a third-generation IL-1RAP CAR, an iCASP9 suicide gene, and a truncated CD19 surface gene. We demonstrated that primary AML blasts can be easily transduced (74.55 ± 21.29%, n = 4) and that CAR T cytotoxicity to IL-1RAP is inversely correlated with epitope masking in relation to the number of antigenic sites expressed on the surface of IL-1RAP+ lines. Importantly, we showed that, in vitro, a 24-h exposure of IL-1RAP+/CAR+ leukemia lines to Rimiducid eliminated >85% of the cells. We confirmed that the expression of IL-1RAP CAR by an IL-1RAP+ leukemic cell, by decreasing the membrane availability of the targeted antigen, can induce resistance while a high epitope density maintains sensitivity to CAR T cells. Moreover, the presence of the iCASP9/Rimiducid suicide system safety switch makes this immunotherapy approach safe for application in a future phase 1 clinical trial.

Background
Innovative adoptive chimeric antigen receptor (CAR) T cell therapy has demonstrated accepted and impressive results in the treatment of hematologic B malignancies, particularly in refractory/relapse (R/R) pediatric or adult acute lymphoid leukemia (ALL) [1] and lymphoma [2] by targeting the CD19 cell surface antigen. Based on these pivotal clinical trials, in which the short-term complete remission (CR) rate has reached 70–90%, two autologous CAR T cell drugs have been approved by the Food and Drug Administration. However, careful retrospective monitoring follow-up has shown that 20–50% of CR patients relapse within the first year and that 10–20% of patients do not achieve CR [3].
Intention-to-treat analysis has revealed several major factors impairing CAR T cell immunotherapy [4]. The first is CAR T cell manufacturing failure, starting from a low-quality apheresis harvest of T cells, either in too small quantities or of inappropriate intrinsic quality from patients who have received multiple treatments depending on the disease’s history, and with production delay that mismatches the progression of the patient’s acute disease [5].

The second problem is primary resistance, independent of target antigen expression (antigen-positive relapse), which affects CAR T cell efficiency early after infusion due to CAR T cell persistence (costimulatory domains), T cell subpopulation composition (naive versus memory T cells), immune checkpoint expression, and negative interaction with regulatory T cells or myeloid-derived suppressor cells [6, 7].

The third reason is relapse, mainly due to loss of membrane antigen target expression (antigen-negative relapse, Ag−/R). Several mechanisms have been identified as immune pressure inducing a lineage switch from ALL to a myeloid phenotype that occurs in MLL-rearranged B-ALL [8], including homozygous mutations of the B cell receptor protein complex [9]; splicing or mutations, respectively, of the CD19 mRNA or gene [10–12]; and tumor/CAR T cell membrane exchange that decreases antigen density and induces fraticidal CAR T cell killing (trogocytosis) [13]. Another reported but unusual Ag−/R CAR T cell escape mechanism is epitope masking following unintentional viral transduction of a single leukemic B cell during CAR T cell production, leading to autorecognition of the target by tumor-expressed CAR, which makes it invisible to the CAR T cells [14].

There are various ways to overcome antigen escape and avoid failure in CAR T cell immunotherapy [15], such as improving CAR T cell production (using interleukin (IL)-7 and IL-15 rather than IL-2), combining the therapy with anti-checkpoint inhibitor antibodies, targeting an alternative cell surface epitope (i.e., CD20, CD22, etc.) expressed by the same tumor cells using dual- or tandem-specificity CARs [16], using a universal CAR [17], or arming the CAR construct to enhance its antitumor activity [18].

Regarding epitope masking, anti-CAR-CAR T cell therapy was recently proposed as an antidote in order to eliminate transduced leukemic B cells [19]. Although useful for treating this unintended consequence of CD19 CAR T cell strategies, it is not applicable to CAR T cells directed against other targets, and much effort and time are required to implement such an autologous approach. Thus there is a need to explore other strategies. Suicide gene safety switches [20, 21] may be a universal and interesting solution to explore while it can also secure at the same time either adverse events due to CAR T cells or but also unexpected and accidental tumor cell transduction.

We present a proof of concept for a CAR T cell immunotherapy approach to targeting chronic myeloid leukemia (CML) or acute myeloid leukemia (AML) leukemic stem cells that express interleukin-1 receptor accessory protein (IL-1RAP) [22]. The method takes advantage of the inducible caspase 9 (iCAS9) suicide gene [23] in our lentiviral construct. Since we plan to use this immunotherapy in the clinic, we modeled the epitope masking and studied the usefulness of the suicide safety switch in the context of AML.

Methods

Patient samples, healthy donor blood samples, and cell lines

Samples were collected from patients with AML who are included in the French Innovative Leukemia Organization Cell Biobank, AML collection (No. BB-0033-00073, declaration 2009-944 and authorization AC 201261739). Peripheral blood mononuclear cells were isolated by Ficoll gradient density centrifugation using Ficoll–Paque from anonymous blood samples collected from healthy donors at the French Blood Center (Besançon, France). Patients and donors provided written informed consent, and the study was conducted in accordance with the ethical guidelines (Declaration of Helsinki) and approved by the local ethics committee of the CPP-Est (CPP2019-03-022, France).

Human tumor cell lines KU812 (CRL-2099), K562 (CCL-243), HEL (ACC-11), and MA9-RAS were kindly provided by James C. Mulloy, Division of Experimental Hematology and Cancer Biology, Cincinnati Children’s Hospital Medical Center, University of Cincinnati College of Medicine, MOLM-13 (ACC-554), MONO-MAC-6 (ACC-124), EOL-1 (ACC-386), HL-60 (UMR645), THP-1 (ACC-16), and 293T (CRL-3216) were obtained from the ATCC or DMSZ and stored in our local master cell bank. A derived working cell bank was use for all experiments in this work.

IL-1RAP CAR T and AML primary cell identification by flow cytometry

Leukemic cells from patients with AML were tracked using a panel containing the following antibodies: CD45-V50 (HI30, BD Biosciences), CD34-V450 (8G2, BD Biosciences), CD38-APC (HB-7, BD Biosciences), CD33-PerCP-Cy5.5 (P67-6, BD Biosciences), CD14-APC-H7 (MqP9, BD Biosciences), and our murine fluorescein isothiocyanate (FITC)-labeled IL-1RAP monoclonal antibody (mAb), clone #A3C3. Transduced cells were stained using antibodies CD3-vioblue (clone REA613, Miltenyi Biotec) and CD19-APC (LT19, Miltenyi Biotec). Cells were collected using a CANTO II cytometer (BD Biosciences) and analyzed using the DIVA 6.1 software (BD Biosciences).
Surface IL-1RAP antigen quantification by flow cytometry

K562, KU812, HEL, HL-60, THP-1, MA9RAS, Molm-13, EOL-1, and Mono-Mac-6 cells were stained for indirect immunofluorescence with specific mAbs and anti-IL-1RAP and analyzed by quantitative flow cytometry. The saturating concentration of primary antibody was previously determined for each cell line according to the manufacturer’s instructions.

IL-1RAP expression levels were determined using indirect immunofluorescence flow cytometry using the Cell Quant Calibrator (Biocide, France). Briefly, cells were labeled with unconjugated anti-IL-1RAP mAb and the binding of the primary antibody was revealed using a FITC-coupled secondary antibody, anti-IgG1 (clone X-56, Miltenyi Biotec). Mean of fluorescence intensity (MFI) calibration values for the calibration beads and the corresponding numbers of mAb molecules were determined. The MFI value of the test sample was compared with the calibration curve to determine the number of bound mAb molecules.

Lentiviral constructs and cell transduction by IL-1RAP CAR vector

The IL-1RAP CAR lentiviral construct was generated as previously described [22]. Briefly, this vector carries a third-generation CAR, an iCASP9 (inducible caspase 9) suicide gene, and a gene encoding surface-expressed delta CD19 for monitoring transduction efficiency and CAR T cell follow-up. The mock control vector does not contain the CAR sequence.

Transduced cells were established from T cell lines, primary T cells from healthy donors, or primary cells from AML patients and transduced with lentiviral supernatant from IL-1RAP CAR- or mock-transduced cells. IL-1RAP CAR T cells, mock-transduced T cells, and untransduced T cells were then used in functionality tests, in order to subtract alloreactivity effect that may occur.

Briefly, T cells were activated using CD3/CD28 magnetic beads (Life Technologies) at a 1:1 ratio in X-vivo15 medium (Lonza) with 500 UI/mL of IL-2 (Proleukin, Novartis) for 2 days, transduced with the CAR-expressing lentivirus, and finally expanded for up to 10 days. CD3+ cells were selected using CD3/CD28 magnetic beads. AML primary cells or cell lines were transduced prior to CD3/CD28 activation or selection.

For all transduced cells, the transduction efficiency was established by use of CD19 staining with an allophycocyanin (APC)-conjugated anti-CD19 antibody (clone LT19, Miltenyi Biotec) and flow cytometry. CAR cell-surface expression was determined using a biotinylated IL-1RAP protein, phycoerythrin-conjugated streptavidin (Miltenyi Biotec), and flow cytometry (BD CantoII).

In vitro CAR T cell cytotoxicity analysis

Untransduced-, mock-transduced, or IL-1RAP CAR T cells were labeled with the cell proliferation dye eFluor 450 (ThermoFisher, Switzerland) following the manufacturer’s protocol. Labeled cells were cultured at an appropriate effector-to-target (E:T) ratio with untransduced, mock+, or CAR+ target AML cell lines at 37 °C for 24 h. After coculture, cells were labeled with 7-aminoactinomycin D (7-AAD; Beckman Coulter, USA) and anti-CD19-APC to evaluate the number of dead (7-AAD positive) target cells.

IL-1RAP tumor xenografted murine models

A xenograft NOD.Cg-Prkdcscid Il2rgtm1Wjl Tg (CMV-IL3, CSF2, KITLG) NSG-SGM3 (NSGS) murine model was used to study CAR T cell cytotoxicity and iCASP9/Rimiducid suicide gene efficiency. NSGS mice were transplanted (intravenously (i.v.)) with bulk Luc+ IL-1RAP+ AML cells that were unmodified or modified with IL-1RAP CAR vector.

HL-60 and IL-1RAP CAR+ HL-60 AML cell lines were transduced with a luciferase lentiviral vector (pLenti CMV V5-Luc Blast vector, Addgene), and luciferase-positive cells were selected by resistance to blasticidin (ThermoFisher).

Six-to-8-week-old NSG-S mice (Jackson Laboratory) were sublethally irradiated (250 cGy) on day −4. On day −3, each mouse received 10^6 HL-60 Luc+ or HL-60 Luc+/CAR+ cells resuspended in 300 µL of phosphate-buffered saline (PBS) by injection into the tail vein. After tumor establishment (day 0), mice were treated with untransduced or IL-RAP CAR T cells (10^7 cells resuspended in 300 µL of PBS) via the tail vein. An untreated group was used as a control. Tumor development was monitored every week, using an IVIS Lumina III system (PerkinElmer) after injection of 3 mg of luciferin (VivoGlo Luciferin, Promega, USA) intraperitoneally (i.p.) within 10 min of imaging.

Mice were monitored until the animals in the untreated group reached a moribund state and manifested signs of leukemia (i.e., weight loss >15%, decreased activity, and/or hind limb paralysis). Animal protocols were performed under the control of the Animal Care and Use Committee of the University of Besançon (CELEAG and protocol 11007R, Veterinary Services for Animal Health & Protection).

In vitro and in vivo iCASP9 safety switch functionality

In order to test the functionality of the iCASP9 safety switch, IL-1RAP CAR-positive AML cell lines or AML patient-derived xenografted (PDX) blasts were cultured in medium...
alone or containing 20 nM of dimerizer (AP1903, Rimiducid, Bellicum Pharmaceutical, Houston, USA). Untransduced cell lines were used for controls. After 24 h, cells were transferred to Trucount tubes (BD Biosciences) and stained with anti-CD19-APC, anti-annexin-V-FITC (Miltenyi), and 7-AAD for flow cytometry. Fluorescence analysis was gated on CD19-positive cells. Cells were considered viable when they were negative for both annexin-V and 7-AAD. The quantification was performed as previously described [23].

To evaluate the functionality of the suicide gene in vivo, mice engrafted with HL-60 or HL-60 CAR+ cells were treated with Rimiducid (50 µg, i.p.) [24]. Control mice received a dose of PBS. Mice were then imaged as described above.

Results

Primary AML leukemic cells expressing different levels of IL-1RAP can be easily transduced using IL-1RAP lentiviral vector supernatant

Primary AML blasts express different levels of IL-1RAP [25]. We confirmed that finding on primary AML blasts (n = 30) using cytometry with our own monoclonal antibody (#A3C3) used for CAR design. Samples representing European Leukemia Net classification groups equally (n = 10 in each group) expressed different levels of IL-1RAP as shown in a representative experiment and as reported using relative fluorescence intensity (RFI). IL-1RAP low expressers are defined by overlapping isotype control histogram (Fig. 1a).
In the absence of T cell selection, leukemic AML primary cells could be transduced by our IL-1RAP CAR and mock vectors with a high efficiency of transduction (71.9 ± 17.6% and 63.5 ± 18.66% for CAR and mock vectors, respectively, n = 6; Fig. 1b, c).

Moreover, the intensity of IL-1RAP expression was slightly decreased on the surface of leukemic AML primary cells transduced by the CAR vector and remained unchanged after transduction with the mock vector in comparison with untransduced primary blast cells (Fig. 1d, upper).

IL-1RAP cell surface expression decrease was also shown on primary blasts (low and high IL-1RAP expressers, n = 6) from AML patients, with a clear difference in high IL-1RAP expressers compared to low IL-1RAP expressers (Fig. 1d, lower).
Lentiviral IL-1RAP CAR transduction of AML cell lines expressing different levels of IL-1RAP affects IL-1RAP detection

In order to model CAR transduction of various cells expressing surface IL-1RAP, we used IL-1RAP RFI and absolute number of antigen site determination to screen different AML cell lines for IL-1RAP epitope expression. We classified AML cell lines into three types: low (<1000), intermediate (>1000 and <2000), and high (>2000), IL-1RAPlow, IL-1RAPinter, and IL-1RAPhigh, respectively) according to the quantification of IL-1RAP antigenic sites by flow cytometry (Fig. 2a). For further experiments, we selected HL-60 (IL-1RAPlow), Molm-13 (IL-1RAPinter), and Mono-Mac-6 (IL-1RAPhigh) cell lines as representative models of the different levels of IL-1RAP expression. As shown in Fig. 2b, all three selected cell lines were transduced with the same efficiency.

The autocrine expression of CAR on the surface of cell lines after lentiviral transduction was confirmed by flow cytometry using the biotinylated IL-1RAP protein for all CAR+ AML cell lines (Fig. 2c, right up). In order to exclude a differential effect due to different transduction efficacy, normalization was performed by gating on subcellular population expressing similar CD19 cell surface. Interestingly, by comparison with untransduced cell lines, the intensity of IL-1RAP signal at the cell surface decreased significantly after CAR expression on HL-60 [fold change (FC) = 0.67; p < 0.01] on CAR+ Molm-13 (FC = 0.67; p < 0.01), and CAR+ Mono-Mac-6 (FC = 0.52; p < 0.01) transduced cells, while it remained stable between mock- transduced and their respective untransduced AML cell lines (FC = 0.98; 0.92, and 1.07, respectively, for HL-60, Molm-13, and Mono-Mac-6) (Fig. 2c, right down and Fig. 2d).

The cytotoxicity of CAR T cells against AML cell lines is reduced only against an IL-1RAPlow-transduced leukemic AML cell line

The cytotoxicity of IL-1RAP CAR T cells was tested in vitro against the three AML cell lines HL-60, Molm-13, and Mono-Mac-6 transduced or not with mock or IL-1RAP CAR vectors. Compared with mock-T cells, after 24 h of coculture at different E:T ratios, IL-1RAP-CAR T cells induced an equivalent mortality in IL-1RAPhigh and IL-1RAPlow CAR+ AML cell lines (88 and 76%, respectively), while the IL-1RAPlow CAR+ AML cell line was significantly less sensitive (57%) even when increasing at an E:T ratio of 5:1 (Fig. 3).

Because the IL-1RAPlow CAR+ HL-60 AML cell line was insensitive to CAR T cell cytotoxicity, we studied the in vivo activity of IL-1RAP CAR T cells against an IL-1RAP Luc+ or Luc+/CAR+ IL-1RAPlow HL-60 AML cell line in a xenografted NSG-S mouse model that received an injection of either HL-60-Luc+ or HL-60 CAR+ Luc+ cells. Compared with the untreated mice or those treated with T cells, mice treated with IL-1RAP CAR T cells displayed a decrease in leukemic burden of untransduced HL-60 cells, leading to complete elimination after day 7. In contrast, in mice that received IL-1RAP CAR+ HL-60 cells, no anti-leukemic effect of CAR T cells was detected in either mock-T cell- or IL-1RAP-CAR T cell-treated mice (Fig. 4).
unmasked antigen sites expressed at the cell surface to render the cells sensitive to killing by the CAR T cells.

A safety switch (suicide gene system iCASP9/\textit{Rimiducid}) allows resistant CAR-transduced AML IL-1RAPlow cells to be eliminated, \textit{in vitro} and \textit{in vivo}

The IL-1RAP CAR vector includes an iCASP9 transgene. We took advantage of the insensitivity of IL-1RAP\textsuperscript{low}-transduced IL-1RAP CAR AML cells to killing by the CAR T cells to test the usefulness of the safety switch in this model of epitope masking. For an \textit{in vivo} test, NSG-S mice were engrafted with only IL-1RAPlow-transduced IL-1RAP CAR HL-60 AML leukemic cells \textit{i.v.} on day 0. AP1903 or PBS was administered \textit{i.p.} to the treatment and control groups, respectively, on day 3 (Fig. 4). As expected, the HL-60 AML tumor is controlled by IL-1RAP CAR T cells, whereas AML IL-1RAP CAR+ HL-60 tumor is not. In addition, if AP1903 treatment alone has no effect on untransduced HL-60 cell line and progressed, the AML HL-60 CAR+ tumor decreased in size after AP1903 injection until it disappeared. Leukemic transduced cells were not eliminated after PBS treatment (data not shown).

In vitro, a 24-h AP1903 (Rimiducid) treatment of IL-1RAP\textsuperscript{low} CAR+ HL-60, IL-1RAP\textsuperscript{inter} CAR+ Molm-13, IL-1RAP\textsuperscript{high} Monomac-6 AML cells, and CAR+ IL-1RAPlow,inter or high PDX AML blasts eliminated >85% of the cells (Fig. 5a, b), independently of IL-1RAP expression levels.

These results indicate that AP1903 or Rimiducid treatment induced effective and significant elimination of the transferred transduced tumor cells by activation of the iCASP9 suicide gene safety switch.

\textbf{Discussion}

CAR T cell immunotherapies have proven to be remarkably efficient in treating B hematological malignancies in R/R patients by targeting the CD19 antigen. This success has been emphasized because it offers an alternative to patients at a therapeutic impasse until probable long-term remission. However, it should be noted that a percentage of patients are refractory to CAR T cells early in treatment or relapse late [26]. These failures can be assigned to intrinsic (expression of non-human transgenes, T cell population subtypes, kind of activating signal) or extrinsic (interaction with microenvironment) factors affecting CAR T cell efficiency. Tumor cells can also develop mechanisms to escape from the cytotoxicity of engineered T cells, remaining antigen positive (when they express an immune checkpoint or disrupting death receptor signaling [27]).
or becoming antigen negative through CD19 splicing or mutations, lineage switching, or trogocytosis. Ruella et al. [14] described and explained escape due to a single B-ALL tumor cell transduction event leading to membrane expression of CAR and consequent auto-masking of the cell surface antigen by the expressed CAR itself, which blocked tumor cell recognition by the CAR T cells. There are several molecular and pharmaco logical on/off safety switches for CAR T cell immunotherapies that reversibly or irreversibly affect CAR T cells [28], but they cannot overcome adverse events involving tumor cells.

Today, major CAR T cell therapies are autologous, and effector cells are usually harvested from the patient at the time of relapse and thus include a large number of tumor cells [29]. Collecting a large number of tumor cells in cytapheresis increases the risk of unintentionally genetically modifying them, after which they may persist throughout the manufacturing process and be infused into the patient. To monitor the presence of tumor cells, the use of highly powerful cytometry or molecular assays used to detect minimal residual disease will be essential in order to identify residual tumor cells at the end of the production process.

Ex vivo manufacturing conditions using medium suitable for T cell activation and expansion but unfavorable for tumor cell growth can limit the occurrence of this unexpected adverse transduction. However, lentivirus vectors can efficiently transduce non-proliferating or slowly proliferating cells, such as CD34+ stem cells [30]. A better selection of starting biological product using either anti-CD3/CD28 bead activation for CD3+ cell selection or specific immunomagnetic CD4+ and CD8+ sorting [31] will avoid bringing tumor cells into the production process.

In the case of epitope masking after tumor cell transduction, there are several options available to overcome antigen loss and circumvent immune escape from CAR T cell therapies. First,
multiple antigen targeting will reduce the probability of both epitope masking, using two different CARs that are co-administered or cotransduced, or encoded in a bicistronic construct [32], or by using a two-antigen-binding domain carried by the same construct with or without T cell activation domain dissociation (tandem CAR T cells) [16]. Another option proposed by Ruella et al. consists of a cellular antidote anti-CAR involving a second CAR targeting the first anti-CD19 scFv expressed by both the T cell effectors and the transduced tumor cells [19]. However, this attractive approach can be applied only to the approved CD19 CAR, which lacks safety switches, and would be difficult, time-consuming, and expensive to implement.

In this work, we showed that epitope masking may occur in AML and demonstrated the usefulness of the suicide iCASP9/AP1903 system in this context, applying it to our original immunotherapy approach targeting IL-1RAP expressed by CML and AML leukemic cells [22]. While it is known that AML leukemic cells express different levels of IL-1RAP [25], by modeling using cell lines we were able to demonstrate that epitope masking occurs on cells expressing relatively low levels of the target, which can effectively be bound up by the CAR expressed on the membrane (total epitope masking). In other cases, the expression of antigen molecules at the cell surface is greater than CAR expression (partial epitope masking), and enough free antigen remains to trigger IL-1RAP CAR T cytotoxicity against the leukemic cells (Fig. 6).

For total epitope masking, we demonstrate that an on-board lentiviral construct safety switch, such as a suicide gene like iCASP9/AP1903 [20, 23], αEGFR/Cetuximab [33], or CD20/Rituximab [34], can eliminate CAR-transduced tumor cells that escape CAR T cell cytotoxicity. The advantage of a suicide gene safety switch is double, because it can also selectively ablate the CAR T cells when adverse events such as cytokine release syndrome, persistent neurotoxicity, on-target/off-tumor responses, or anaphylaxis occur.

**Conclusions**

In conclusion, IL-1RAP CAR T cells produced from CD4+ and CD8+ selected cells and carrying an iCASP9 safety
switch will strengthen the safety of our CAR T cell immunotherapy and will be applied in a future phase I clinical trial in AML.

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**Fig. 6** Summary model of the iCASP9 safety switch in autologous AML tumor escape induced by epitope masking after unexpected transduction of leukemic cells. Transduced IL-1RAP CAR+/IL-1RAP antigen-expressing AML tumor cells are eliminated by IL-1RAP CAR T cells when the number of IL-1RAP antigen sites remains greater than the number of IL-1RAP CAR+ surface molecules (partial epitope masking). In case of total epitope masking after CAR transduction of AML tumor cells expressing low levels of IL-1RAP antigen, AP1903 (Rimiducid) exposure eliminates the cells that are resistant to CAR T cell immunotherapy.

**Author contributions** WW, MD, CF, and MNDR formulated the initial idea and designed and planned the study. RT, MNDR, LB, and CN performed in vitro experiments, analyzed data, and contributed to the figures. WW and RH performed in vivo animal experiments and analyzed the data. XR and YS contributed to the manuscript. WW and CF wrote the manuscript. All authors reviewed the manuscript and contributed to its improvement.

**Compliance with ethical standards**

**Conflict of interest** CF: consulting for Novartis, BMS, Incyte, and Daichii; research grant from Novartis.

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