SAR3419: An Anti-CD19-Maytansinoid Immunoconjugate for the Treatment of B-Cell Malignancies

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Abstract

SAR3419 is a novel anti-CD19 humanized monoclonal antibody conjugated to a maytansine derivate through a cleavable linker for the treatment of B-cell malignancies. SAR3419 combines the strengths of a high-potency tubulin inhibitor and the exquisite B-cell selectivity of an anti-CD19 antibody. The internalization and processing of SAR3419, following its binding at the surface of CD19-positive human lymphoma cell lines and xenograft models, release active metabolites that trigger cell-cycle arrest and apoptosis, leading to cell death and tumor regression. SAR3419 has also been shown to be active in different lymphoma xenograft models, including aggressive diffuse large B-cell lymphoma, resulting in complete regressions and tumor-free survival. In these models, the activity of SAR3419 compared favorably with rituximab and lymphoma standard of care chemotherapy. Two phase I trials with 2 different schedules of SAR3419 as a single agent were conducted in refractory/relapsed B-cell non-Hodgkin lymphoma. Activity was reported in both schedules, in heavily pretreated patients of both follicular and diffuse large B-cell lymphoma subtypes, with a notable lack of significant hematological toxicity, validating SAR3419 as an effective antibody-drug conjugate and opening opportunities in the future. Numerous B-cell–specific anti-CD19 biologics are available to treat B-cell non-Hodgkin lymphoma, and early phase I results obtained with SAR3419 suggest that it is a promising candidate for further development in this disease. In addition, thanks to the broad expression of CD19, SAR3419 may provide treatment options for B-cell leukemias that are often CD20-negative. *Clin Cancer Res; 17(20); 6448–58. ©2011 AACR.*

Introduction

Non-Hodgkin lymphoma (NHL) is the most common lymphoma, with an estimated 65,540 new cases and 20,210 deaths registered in the United States in 2010 (1). NHL originates from the malignant development of B or T lymphocytes and comprises a heterogeneous group of malignancies (2), among which B-cell lymphomas account for 85% of all cases (3). Despite the significant improvement in overall survival of patients with both aggressive and indolent B-cell NHL that has been achieved following the addition of rituximab to conventional chemotherapy (4), not all patients who have CD20-positive tumors respond to the treatment (5, 6), and most patients who have disease response will relapse (7). More than 50% of patients with NHL will die of their disease (4–8). Since 2005, 5 new drugs have been approved for the treatment of lymphoma (6, 9), including 2 for B-cell NHL, namely, bortezomib, a proteasome inhibitor approved for the treatment of mantle cell lymphoma in 2006, and bendamustine, a DNA alkylating and antimetabolite agent approved for the treatment of indolent B-cell NHL in 2008. However, despite the large number of trials of novel agents, including monoclonal antibodies and small-molecule inhibitors (3, 10), there is still an important unmet medical need, particularly in second-line and subsequent lines of therapy. Concerning monoclonal antibodies, different modalities of conjugation have been tested in the clinic and have shown responses in hematological malignancies and NHL in particular, as discussed in this *CCR Focus* section, dedicated to antibody conjugates (11). These modalities include radionuclide conjugation (12), toxin conjugation (13), and small-molecule drug conjugation, also called antibody-drug conjugates (ADC; refs. 14–16). ADCs represent the most active field today, with at least 20 such ADCs in development (11). Very promising clinical responses have been achieved in both solid tumors, as shown by T-DM1 for the treatment of HER2-positive breast tumors (14), and hematological malignancies, as shown by SGN35 (15) and CMC-544 (16) for Hodgkin lymphoma and NHL, respectively.

Monoclonal Antibodies beyond Rituximab for Treating B-Cell Malignancies

Since the introduction of rituximab in 1997, only 2 more "naked" (nonconjugated) antibodies have been approved for the treatment of lymphoma: (i) alemtuzumab, an

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anti-CD52 antibody approved in 2001 for the treatment of CLL (17); and (ii) ofatumumab, a second-generation anti-CD20 antibody displaying increased complement-dependent cytotoxicity compared with rituximab, that was approved in 2009 for relapse/refractory CLL patients who failed fludarabine and alemtuzumab (18). In addition to these 2 naked antibodies, 2 murine anti-CD20 radionuclide conjugates were approved for consolidation treatment in 2002 (^{90}Y-ibritumomab) and 2003 (^{131}I-tositumomab; refs. 12 and 19).

In recent years, investigators have made a considerable effort to further improve NHL treatment, and several second- and third-generation anti-CD20 monoclonal antibodies have been examined in clinical trials (20). In addition, exploration of other B-cell–specific antigens, including CD19, CD22, CD37, and CD40, as potential targets for the development of new antibody-based treatments is growing. Antibodies are being investigated as naked molecules, such as the anti-CD22 epratuzumab (21), the anti-CD40 dacetuzumab (22), and the different anti-CD19 antibodies (Table 1), or in an engineered format, such as the anti-CD19/anti-CD3–bispecific blinatumomab (23) and the anti-CD37–specific small modular immunopharmaceutical TRU-016 (24). The advent of ADC technologies has also led to clinical evaluation of conjugated antibodies, such as the anti-CD22-calicheamicin conjugate CMC544 (16) and the subject of this review, the anti-CD19-maytansinoid conjugate SAR3419 (25–28).

CD19 Antigen

CD19 is a type I transmembrane glycoprotein of the immunoglobulin Ig superfamily, with expression restricted to B cells (29). CD19 is involved in B-cell fate and differentiation through the modulation of B-cell receptor signaling at multiple stages of B-cell development (29, 30). CD19 is ubiquitously expressed on B cells (25), as it is found expressed from the early pre-B stage throughout B-cell differentiation up to mature B cells, before it is downmodulated at the plasma cell stage (Fig. 1). Thus, CD19 has broader expression than CD20. The pattern of CD19 expression is maintained in B-cell malignancies, covering all subtypes of B-cell lymphoma, from indolent to aggressive forms, as well as B-cell chronic lymphocytic leukemia and non-T acute lymphoblastic leukemia (31–33), and allows the targeting of tumor indications of early B cells, such as acute lymphoblastic leukemia (ALL), which cannot be targeted by rituximab. The quantification of the number of CD19 molecules at the surface of tumor cells and cell lines derived from malignant B cells resulted in a wide range of

Table 1. Clinical trials and discovery projects targeting CD19

Biologic compound	Type	Mechanism of action	Phase (initiation date)
MT-103 (blinatumomab) Micromet, Inc.	Bispecific scFv anti-CD19/anti-CD3 BiTE	T-cell recruitment and activation	I/II/III (2007) Pivotal trial for MDR$^+$ ALL
SAR3419 Sanofi-Aventis/ImmunoGen, Inc.	Humanized anti-CD19 mAb conjugated to maytansinoid DM4	ADC (tubulin binder)	I/II (2007)
MEDI-551 MedImmune/ Astra-Zeneca	Glycoengineered humanized anti-CD19 mAb (BioWa's Potelligent)	Naked antibody high-affinity FcγRIII-enhanced ADCC	I (2010)
MOR-208/XmAb5574 Xencor/Morphosys	Fc engineered humanized anti-CD19 mAb	Naked antibody high-affinity FcγRIII-enhanced ADCC	I (2010)
MDX-1342 Medarex/ Bristol-Myers Squibb	Glycoengineered fully human anti-CD19 mAb (BioWa's Potelligent)	Naked antibody high-affinity FcγRIII-enhanced ADCC	I (2008, on hold)
Combotox University of Texas Southwestern/Abiogen	Mixture of chimeric anti-CD19 mAb HD37 and anti-CD22 mAb RFB4, both conjugated to deglycosylated ricin A-chain (HD37-dgA + RFB4-dgA)	Immunotoxin conjugate with deglycosylated ricin A-chain	I (2005)
DI-B4 Merck KGaA/Cancer Research UK	Chimeric anti-CD19 mAb monoclonal antibody	Naked antibody ADCC	I (2010)
SGN-19A Seattle Genetics	Fully human anti-CD19 mAb (hBU12) conjugated to auristatin (vc-MMAE)	ADC (tubulin binder)	Discovery
MDX-1206 Medarex/ Bristol-Myers Squibb	Fully human anti-CD19 mAb (MDX1435) conjugated to duocarmycin (vc-MGBAA)	ADC (DNA alkylating agent)	Discovery
AFM-11 Affimed Therapeutics AG	Tetravalent tandem antibody (TandAb) anti-CD19/anti-CD3	T-cell recruitment	Discovery
AFM-12 Affimed Therapeutics AG	Tetravalent tandem antibody (TandAb) anti-CD19/anti-CD16	NK cell recruitment	Discovery

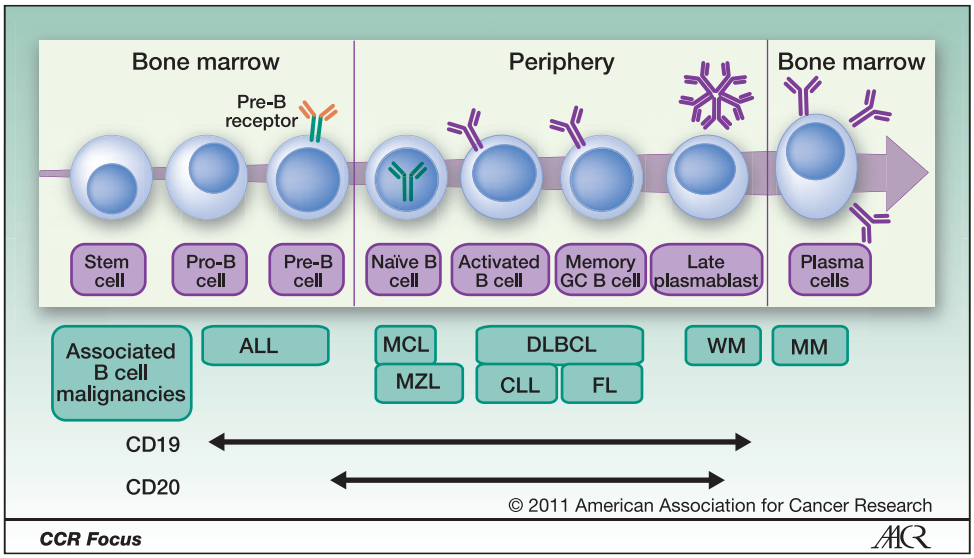

Figure 1. Pattern of expression of CD19 and CD20 antigens during B-cell development and associated malignancies. A simplified cartoon of B-cell lineage, B-cell malignancies, and antigen expression (59, 60). The positioning of the different B-cell malignancies associated with different stages of B-cell development is abridged and illustrative only; a detailed description is beyond the scope of this review.

published values due to differences in cell line clones, the antibodies studied, and the methodologies used. For example, CD19 expression was reported to be both >100,000 sites per cell (34) and ~10,000 sites per cell (35) for the same cellular models. In our hands, using a calibration curve of beads loaded with different amounts of human IgG1, the quantification of CD19 antigen at the surface of Ramos, Raji, and Daudi lymphoma cell lines gave average values of 42,166, 18,315, and 14,077 antibody-binding sites per cell, respectively. With the same assay, the quantification of CD19 on the surface of normal B lymphocytes yielded values in the range of 11,000 to 16,000 antibody-binding sites per cell, an order of magnitude similar to that of the lymphoma cell lines tested.

CD19 was shown to be internalized efficiently in lymphoma tumor models with the use of different antibodies, such as huB4 (35) and hBU12 (36). In our laboratories, we quantified the internalization and processing of the anti-CD19 antibody huB4 using an Alexa488 antibody conjugate and different tumor cell lines upon incubation at 37°C. The kinetics of appearance of antibody-free fluorescence, produced by lysosomal degradation of the antibody moiety, showed internalization and processing of the huB4-Alexa488 in all models, with some variability between models (35) that may reflect differences in the capacity of CD19 to internalize in different cellular contexts. Indeed, the level of CD21 coreceptor, expressed in a subset of lymphomas, was described to affect the internalization and efficacy of an anti-CD19-maytansinoid conjugate through a noncleavable linker (37). However, this observation was not confirmed in a second study using auristatin derivatives conjugated to a different anti-CD19 antibody through a cleavable linker (36).

Anti-CD19 Intervention

CD19's B-cell lineage–restricted expression and moderate to high homogeneous expression in most cases of lymphoma make it an attractive target for therapies for B-cell malignancies, as shown by the many previous and ongoing therapeutic interventions that focus on CD19 (Table 1). Different types of molecules targeting CD19 are being developed that can broadly fit into 3 main classes: (i) naked antibodies, most of which undergo modifications of their Fc portion to enhance binding to FcRγIII and subsequently enhance antibody-dependent cell-mediated cytotoxicity (ADCC) activity, such as MOR-208 and MEDI-551; (ii) bispecific antibodies with one arm binding to CD19 and one arm binding to either the T-cell receptor (as exemplified by blinatumomab, the most advanced anti-CD19 therapeutic molecule) or NK-cell receptors (as in the molecule AFM12, a tetravalent tandem antibody that is in the discovery stage of development); and (iii) antibody conjugates, for which anti-CD19 antibodies are conjugated to either a toxin (as exemplified by the ricin-based immunotoxin Combotox) or a potent low-molecular-weight cytotoxic molecule (as exemplified by SAR3419). As of today, Combotox has shown anticancer activity in NHL (38) and ALL (39) that may warrant further clinical testing. Studies of blinatumomab (23) and SAR3419 (27) have provided a clinical proof of concept, showing a notable rate of objective response in pretreated patients in phase I clinical trials.

SAR3419 Structure and Mechanism of Action

SAR3419 is an ADC that consists of a humanized monoclonal IgG1 antibody (huB4) attached to a highly potent tubulin inhibitor, the maytansinoid DM4 (40), through reaction with an optimized cleavable linker, *N*-succinimidyl-4-(2-pyridyldithio)butyrate (SPDB linker; Fig. 2). The succinimidyl group of the linker reacts with amino groups of lysine residues of the antibody to form stable amide bonds, and the pyridyldithio moiety reacts with the sulfhydryl group of DM4 to form a hindered disulfide bond between the linker and DM4. The disulfide bond can be cleaved inside target cells by thiol-disulfide

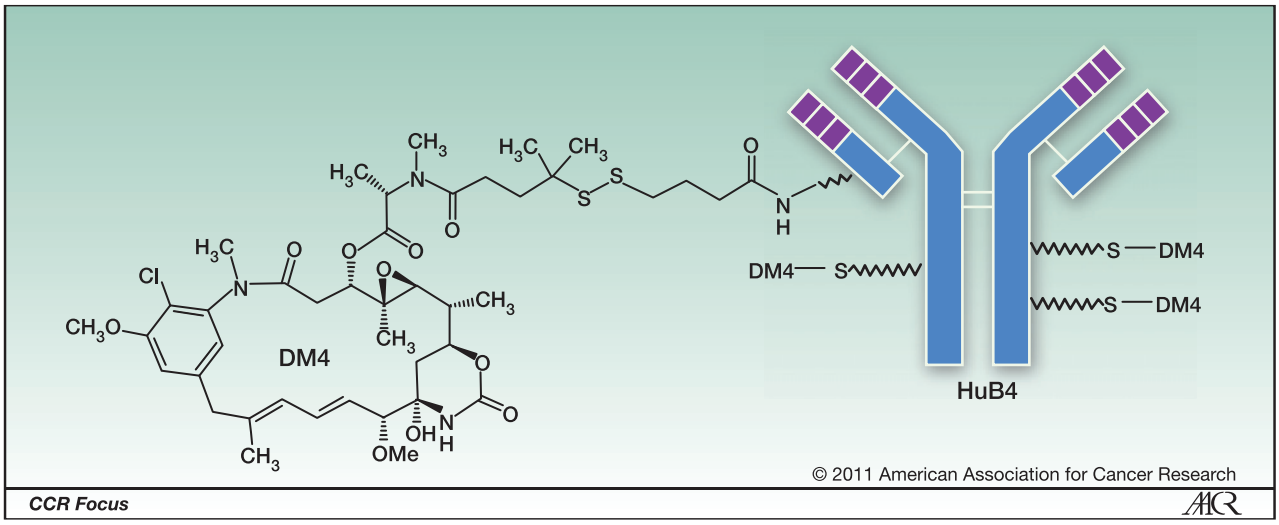

Figure 2. Structure of SAR3419. Figure is adapted from Al-Katib et al. (43).

exchange reactions to release fully active DM4 (28). SAR3419 contains an average of ~3.5 DM4 molecules per molecule of antibody.

The murine B4 antibody, one of the earliest antibodies to define the CD19 antigen (31), was humanized via a variable-domain resurfacing method (41) to yield huB4. The huB4 antibody displays a subnanomolar affinity for CD19 on the surface of B cells, and this affinity is conserved following conjugation to create SAR3419 (35). The huB4 antibody was shown to induce ADCC (data not shown) but not complement-dependent cytotoxicity as described for other anti-CD19 antibodies (42). The ADCC activity was conserved following conjugation to the maytansinoid. The naked antibody has no direct antiproliferative or proapoptotic activity. Therefore, huB4 was not found to be active *in vivo* as a single agent in severe combined immunodeficient (SCID) mice bearing several different lymphomas (43). The huB4 antibody binds only to the human CD19 antigen (29); it does not recognize CD19 in rodent or cynomolgus monkey toxicology models, and thus no B-cell depletion studies have been done with either the huB4 antibody or SAR3419 in animal models.

Maytansine and other maytansinoids are antimitotic agents that bind to tubulin, inhibiting microtubule assembly and inducing G_2/M arrest in the cell cycle, which subsequently leads to cell death (44). Maytansinoids are very potent, displaying cytotoxic activity in the 10 to 90 pmol/L range across several tumor cell lines, including lymphoma lines (45). The SPDB linker was selected based on its superior activity *in vivo* compared with SPP (another cleavable linker with a more labile disulfide bond) and the noncleavable SMCC linker in studies analogous to those described by Kellogg and colleagues (46) for another antibody-maytansinoid conjugate.

SAR3419 was shown to display potent *in vitro* cytotoxicity after 5 days of exposure to different CD19-positive lymphoma cell lines, with EC_{50} values ranging from subnanomolar to a few nanomolars (35). SAR3419 induced a rapid cell-cycle arrest at the G_2/M phase (within the first 24 hours of exposure) followed by an increase in apoptotic cells from 24 to 48 hours (35), as expected for delivery of the maytansinoid payload into CD19+ cells. As has been shown in the case of other maytansinoid conjugates (47) and in experiments with SAR3419 in which the DM4 moiety was radiolabeled (48), the mechanism of action of SAR3419 involves binding to the cell-surface antigen, followed by internalization via endocytosis and subsequent intracellular routing to lysosomes where the huB4 antibody moiety of the ADC is degraded to yield the lysine-SPDB-DM4 metabolite. Once it is in the reducing environment of the cytoplasm, this metabolite is further subjected to cleavage by thiol-disulfide exchange reactions to release the free maytansinoid thiol compound DM4 (47, 48). DM4 is then S-methylated to form *S*-methyl-DM4 by an endogenous S-methyl-transferase (47). The final metabolite of cancer cell metabolism of SAR3419, *S*-methyl-DM4, has a high potency as a microtubule poison that was shown to be similar to the potency of maytansine itself when compared in cytotoxicity assays *in vitro* on different tumor cell lines (40, 44, 49). The early metabolite formed upon lysosomal processing of the ADC, lysine-SPDB-DM4, shows a 1,000-fold lower activity on cell lines *in vitro* resulting from the negative effect of the charged lysine residue on its ability to diffuse across the plasma membrane into cells (47). However, such lysine-linker-maytansinoid species are potent microtubule poisons when they are released inside cells following uptake and lysosomal processing of an ADC (28, 47).

The mechanism of action of SAR3419 was also shown *in vivo* in immunodeficient SCID mice bearing human Ramos Burkitt's lymphoma, a model that was shown to be highly sensitive to SAR3419 (35). In recent studies done in our laboratories, SAR3419 was administered at a single dose of 20 mg/kg 14 days after tumor subcutaneous implantation. Tumor sampling was done at different times, and immunohistochemical evaluation of phospho-histone H3

(*p*-histone H3, a mitosis biomarker) and cleaved caspase 3 (a proapoptotic marker) was done. The single-dose treatment with SAR3419 induced a significant increase in staining for *p*-histone H3, revealing a mitosis blockade that was apparent 24 hours after administration (Fig. 3). This mitosis blockade was then followed by tumor cell apoptosis as evidenced 48 hours after dosing with the ADC by a marked increase in cells that stained positively for cleaved caspase 3 (Fig. 3).

Studies were also done in the same Ramos xenograft model to assess whether the pattern of metabolic processing observed in cell lines was the same as that seen in xenograft models. Mice bearing the tumor xenografts were injected with a single administration of radiolabeled [^{3}H]-SAR3419 (conjugate made with tritiated DM4) at 15 mg/kg in experiments similar to previous experiments involving another ADC with the same linker-maytansinoid design (49). Tumor sampling was done at different time points, and all metabolites were quantified. The total amount of low-molecular-weight (i.e., not protein-linked) metabolites increased rapidly from 2 to 8 hours, with a subsequent linear progression from 8 to 48 hours, and an overall 6-fold accumulation from 2 to 48 hours (Fig. 4). At the latter time point, 71% of the total radioactivity in the tumor xenograft corresponded to low-molecular-weight metabolites, in contrast to just 30% at 2 hours (data not shown). The processing of radiolabeled SAR3419 *in vivo* was found to be similar to the *in vitro* findings (Fig. 4), with the sequential formation of lysine-SPDB-DM4 (the major species at 2 hours) followed by DM4 (about 10% of total nonproteic metabolites at 2 hours, rising to ~24% at 8 hours) and then by the appearance of *S*-methyl-DM4 (first detected at 8 hours). As observed *in vitro*, similar quantities of lysine-SPDB-DM4 and *S*-methyl-DM4 were found in the tumors after 48 hours (Fig. 4; ref. 49). The time course of the formation of the active maytansinoid metabolites in the tumor is consistent with the observed high induction of *p*-histone H3 and cleaved caspase 3 at 24 and 48 hours, respectively (Fig. 3).

SAR3419 Preclinical Activities in Different Lymphoma Models

SAR3419 has shown *in vivo* efficacy in different lymphoma models, including Burkitt's lymphomas (Namalwa, Ramos, and Raji) and diffuse large B-cell lymphoma [DLBCL (RL, WSU-DLCL2, and WSU-FSCCL)] implanted in SCID mice (35, 43). In all models, SAR3419 showed a high level of activity leading to complete responses, with mice being tumor-free at study termination at the highest doses and with significant tumor growth delay at the lower doses (both when injected as a single dose and in a multiple-dose schedule).

As an illustration, in the Ramos lymphoma xenograft model (35), when treated by the intravenous route (2 doses with 4-day interval), SAR3419 was highly active at the dosage of 15 mg/kg (a well-tolerated dosage as evidenced by no loss in body weight), and 100% of the mice were tumor-free at the end of the study (day 124). The lower doses of 7.5 and 3.3 mg/kg were also highly active, with complete tumor regressions in 100% of the animals and with 5 out of 7 mice being tumor-free at the dose of 7.5 mg/kg on day 124. In comparison, treatment with unconjugated DM4 at a dose equivalent to the 15 mg/kg dose of SAR3419 (0.35 mg/kg of DM4, 2 doses with 4-day interval) showed no significant antitumor activity, indicating that conjugation of the DM4 to the antibody was critical for delivering sufficient maytansinoid to the cancer cells *in vivo* to induce tumor shrinkage.

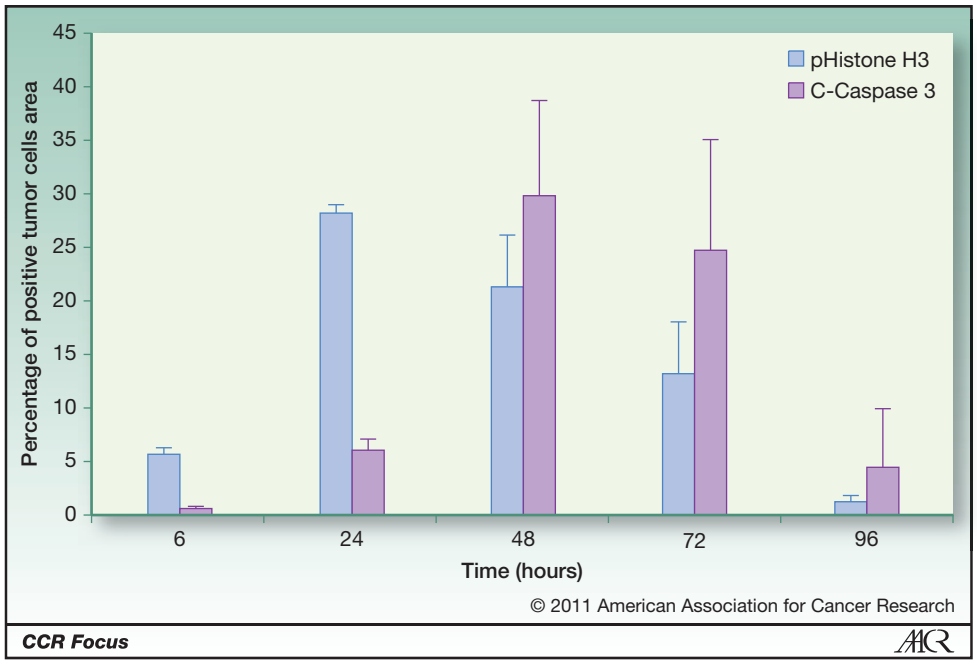

Figure 3. Kinetics of pharmacodynamic markers *p*-histone H3 and cleaved caspase 3 (C-Caspase 3) in Ramos xenograft model. Quantitative evaluation of *p*-Histone H3 and C-Caspase 3 by image analysis quantification at 6, 24, 48, 72, and 96 hours after administration of a single intravenous dose of 20 mg/kg of SAR3419 in SCID mice bearing established Ramos subcutaneous xenograft tumors.

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