# *In vitro* Stability and Immunoreactivity of $^{74}$As[SATA]Vatuximab®

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**Introduction:** Vascular phosphatidylserine (PS) exposure is observed in solid tumors as a result of exposure to stress conditions in the tumor microenvironment [1,2]. Therefore antibodies against PS should be ideal for tumor targeting, especially for the targeting of tumor vasculature endothelium [2]. The Vatuximab® anti-PS antibody (AB) was labelled with the PET isotope $^{74}$As ($T_{1/2}$=17.77 d, 29% $\beta^+$) as described in [3] and tested for its *in vitro* long term stability and immunoreactivity after labelling. Purpose of this study was the evaluation of the developed labelling method for ABs with radioactive arsenic isotopes, using Vatuximab® as an example.

**Experimental:** The antibody was SATA-modified and labelled as described in [3]. The deprotection of the sulfhydryl groups was performed directly before the labelling. 100 µg of SATA-modified antibody in 3 ml PBS at pH=7.5 then were combined with nca [*As]$AsI_3$ solution at 37°C for 30 minutes. Quality control was performed with HPLC, using an Agilent 1100 Series HPLC system, with an LDC/Milton Roy UV-Monitor III at 254 nm and a 'Gabi' NaI-radiation Monitor from Raytest. The column was a Bio-Silect Sec 250-5, 300x7.8 mm and PBS + 0.01M $NaN_3$ was used as solvent.
*In vitro* stability of the radioarsenic labeled ch3G4 was tested via incubation in fetal bovine serum (FBS) and HPLC measurements at various timepoints up to 72 h. 10 µg of radioarsenic labeled ch3G4 in 50 µl PBS were combined with 500 µl FBS and incubated at T= 37°C. Aliquots of 50 µl were taken at t=30 min, 24, 48, and 72 h, diluted with 200 µl water and 20 µl were applied to HPLC under the conditions described above.
The immunoreactivity of the [*As]SATA-Vatuximab® was tested using ELISA, with unlabelled anti-PS as positive control and [*As]SATA-Rituxan® as negative control.

**Results and Discussion:** The quality control showed labelling yields after 30 min labelling time in general were above 99.9 %. The *in vitro* stability of the radioarsenic label was evaluated by incubation in serum, followed by HPLC. No degradation of the label was observed for incubation times up to 72 h. This reflects the covalent bound of the arsenic to the antibody. Attempts by other groups to bind arsenic via complexation to biomolecules always failed because of the low *in vitro* stability due to exchange with free thiols in the blood. The immunoreactivity of the labelled ch3G4 could be demonstrated using ELISA and no inhibition of immunoreactivity through SATA-modification and subsequent labelling with nca *$AsI_3$ could be observed.

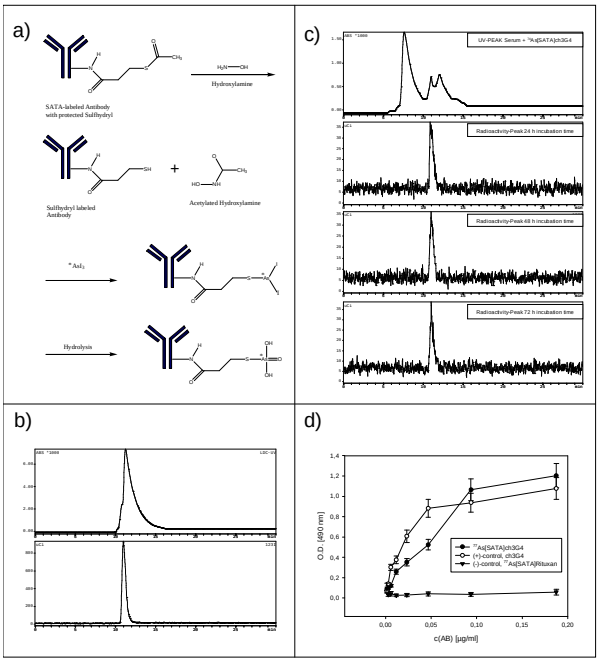


**Fig. 1.**
**a) Reaction scheme for the labelling of SATA-modified antibodies with radioactive arsenic isotopes**. The *$AsI_3$ couples to one SH under elimination of HI, which can be catched by salts in the buffer solution. The 2 iodines remaining at the arsenic are getting hydrolysed and we assume that As is oxidized to +V.

**b) Quality control of the labelling of ch3G4.** After a labelling time of 30 min, an aliquot of 20 µl of the $^{74}$As[SATA]ch3G4-solution was given over a size-exclusion column for radio-HPLC. The upper graph shows the UV-spectrum, the lower the corresponding radioactivity progression. No free $^{74}$As was detectable.
**c) *in vitro* stability of [$^{74}$As]SATA-ch3G4.** The upper graph shows the UV spectrum. Because of the low concentration of AB versus serum-proteins, we detected a typical serum profile. The lower graph shows the corresponding radioactivity peak remaining unchanged in position and peak area.
**d) Immunoreactivity.** Immunoreactivity was tested with an ELISA of $^{77}$As[SATA]ch3G4, using unlabelled and unmodified ch3G4 as positive control and $^{77}$As[SATA]Rituxan as negative control. No reduction of immunoreactivity through the applied SATA-modification with subsequent radioarsenic labeling was detectable.

**Conclusion:** The new method of labelling antibodies with radioactive arsenic isotopes was examplified using the anti-PS AB Vatuximab®. Conditions for standard testing systems were established and evaluated. Labelling reactions were quantitatively and the label was stable attached to the AB, even though incubated in serum for 72 hours. The SATA modification and the used labelling method did not change the ABs immunoreactivity. This *in vitro* results very much encourage future *in vivo* evaluations of radioarsenic labelled antibodies.

**References:**
[1] Ran, S. et al., Cancer Research 62, 6132-6140, 2002
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[3] Jennewein, M. et al., Annual Report, this issue.

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