Status Report for Experiment AD-4/ACE

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I. Introduction

The AD-4 Experiment was proposed to give a solid scientific basis to the notion that antiprotons could be a better tool for controlling localized cancer tumors than protons or heavy ions. This idea is based on three main observations:

1. Compared with protons, the physical dose should be augmented near the end of range due to the additional energy deposited locally when antiprotons annihilate.
2. Some of the additional energy deposited results from low energy ion recoils produced in the annihilation event, which are expected to exhibit a high biological efficiency. Monte Carlo calculations indicate this effect to be from light short-range nuclei.
3. Antiprotons deposit less energy than heavy ions in the entrance channel for an equal dose in the target volume.
4. Pions and high-energy photons generated in the annihilation events can be used to detect in real time the exact location of the treatment.

After running at low energy (50 MeV) for initial investigations of the biological method in 2003 and 2004 we changed to higher beam energy of 126 MeV (500 MeV/c momentum) in 2006 to achieve a clinical relevant penetration depth in a water target. As switching between extraction at 5 MeV and 126 MeV is not a straight forward activity this required a different mode of operation where the beam for the AD-4 experiment is set-up on a Monday and then delivered to the DEM zone 24 hours a day until the morning of following Monday. Normally we split these 168 hours between beam set-up (24 hours), dosimetry checks (48 hours), and biological experiments (96 hours). As the biological data set from the 2011 run was unusable due to a contamination in the cell samples we had requested two weeks (separated by several months) for running for 2012. Ultimately we were only granted 1 week for biological measurements and an additional 3 days of beam time for detector development and dosimetry activities. To make best use of the limited beam time we decided to perform two independent sets of measurements in the one-week run, starting early in the week and reducing the time for set-up and dosimetry. Luckily the cells behaved well this year, as expected, and we obtained two more data sets.

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II. Clonogenic survival experiments

Since we have shifted to the higher beam energy we have collected five independent data sets on clonogenic survival of V-79 Chinese Hamster cells from which we now can attempt to extract the relative biological efficiency (RBE) of antiprotons along the entire beam path from the entrance channel to the Bragg peak and beyond. The main challenge we are facing in this final step of the data analysis is the changing conditions of the experiment and the continuing evolution of the FLUKA Monte Carlo code used for dose planning calculations. For this reason we are not yet prepared to provide a final outcome of the experiment.

For a better understanding of the issues and problems faced by our collaboration in these measurements we will explain the procedures of measurements and analysis in some detail.

Irradiation is performed on Rexolite tubes filled with a mixture of gelatine and V79 Chinese Hamster cells. The cell density is chosen to give 68,000 cells in a 1 mm slice of the 6 mm diameter tube. After irradiation the gelatin is pushed out of the tube slowly and 2 mm thick slices are cut from the extruded gelatin using a thin wire. The slices are dissolved in culture medium and a number of cells, predetermined by using estimates for the dose delivered to a given sample using the Monte-Carlo code FLUKA, are plated into Petri dishes. The number of cells plated is chosen such that for the expected dose approximately 100 viable cells will be plated per dish. This is to allow clonogenic cells to divide and grow colonies over as period of 3 – 5 days. A cell is recognized as clonogenic when it has been able to divide at least 5 times, leading to colonies consisting of more than 50 cells and visible to the naked eye. Too few cells plated would result in low counts of colonies and thus large statistical error bars, too many cells would lead to overcrowding and inhibition of colony development. In the years prior to 2010 plating was done using three different dilution factors (1/3, 1, 3 times the optimal number) as the exact number of cells per slice was not possible to establish with the then available techniques. Since 2010 we are able to use a flow cytometry or FACS (Fluorescence Activated Cell Sorting) setup at the University Hospital in Aarhus. Individual cells held in a thin stream of fluid are passed through one or more laser beams and cause light to scatter and fluorescent dyes to emit light at various frequencies. Photomultiplier tubes (PMT) convert this light to electrical signals and cell data are collected. This enables us to plate an exact number of cells and instead of plating three different dilutions we now plate 4 replicas from each slice to improve statistical analysis.

In the raw data of survival vs. dept in the target we observe a steep increase of cell killing at the proximal edge of a spread-out Bragg peak (SOBP), in contrast to carbon ions, where cell death is increasing more gradually along the beam path (Figure 1). This trend is consistently observed in all data sets, including the two sets collected in 2012 (Figure 2a and b).

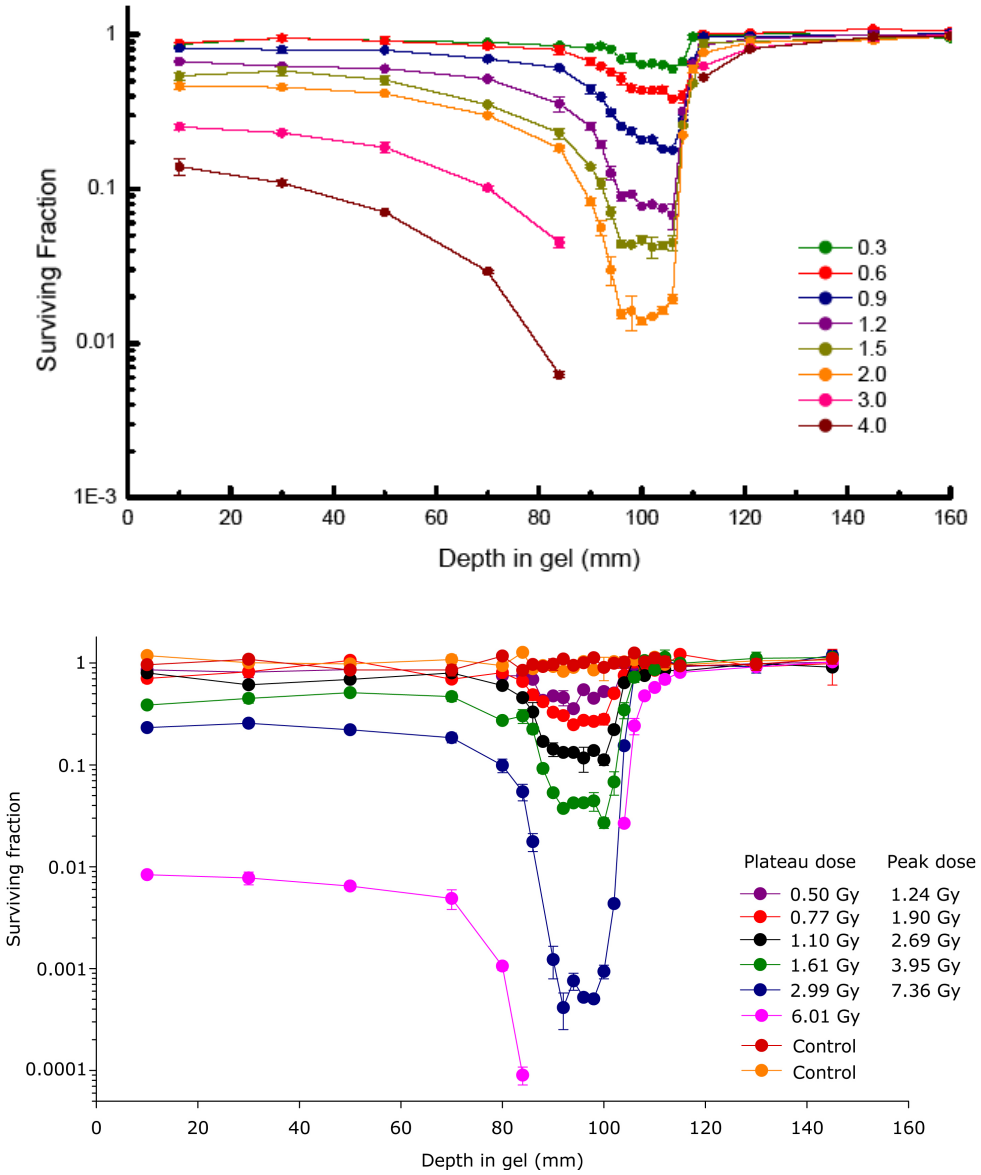

Figure 1: Cell response of V79-WRNE Chinese Hamster cells to irradiation with carbon ions (top) performed at GSI in 2009 and with antiprotons (bottom) from the run in 2008 at CERN.

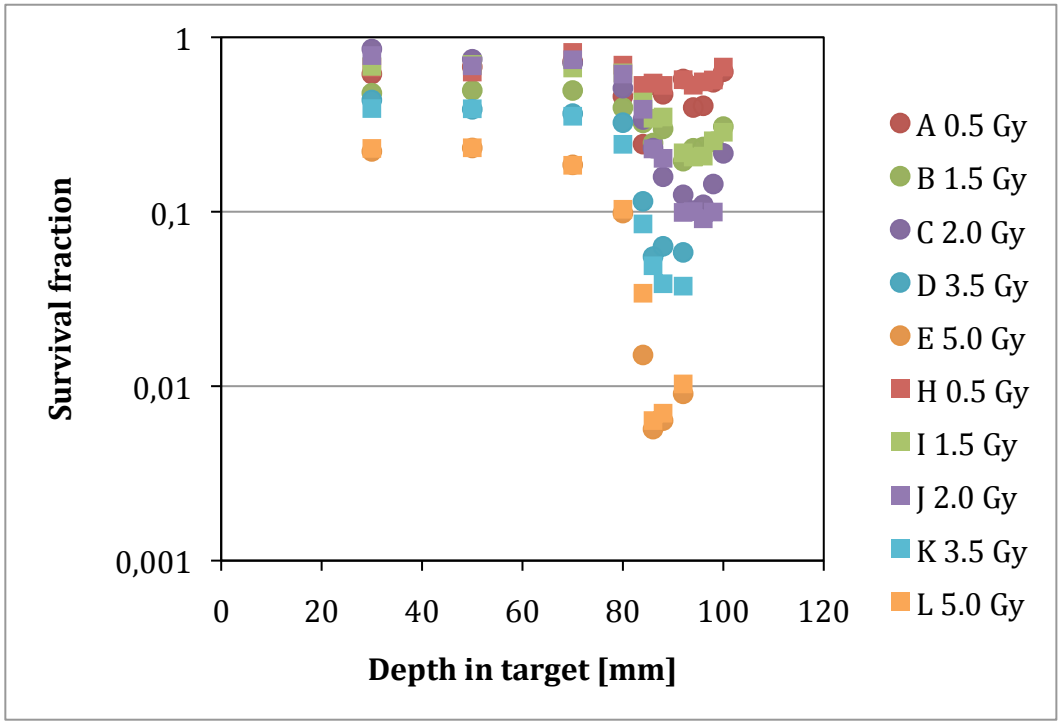

Figure 2: Collective raw data from the 2012 run. Colors indicate specific dose levels and the two different symbols indicate the two separate experiments performed.

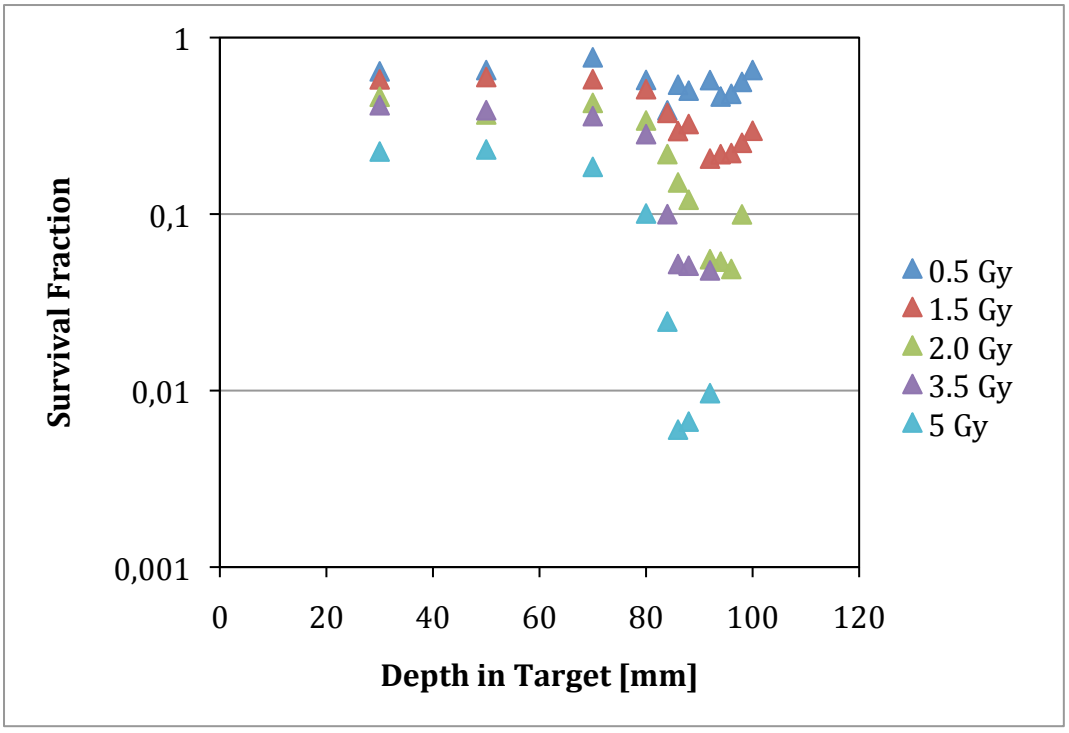

Figure 2b: Clonogenic survival vs. depth for the 2012 run, averaging over the two independent experiments.

In order to normalize the cell counts from the irradiated tubes for other effects influencing the survival rate of the cells, the exact same procedure is also followed for a set of "control tubes" that have been handled identically to the irradiated tubes,

including shipment from Aarhus to CERN and back and storage on ice in the AD-accelerator hall, away from the direct beam, but in the same overall radiation background environment as the irradiated cells. In addition, we kept a number of cell tubes behind in the laboratory at the Aarhus University in order to detect possible effects from the transportation. This can be used to correct the cell counts of the irradiated cells for the 'plating efficiency' which includes all effects on the cells which are independent of the actual irradiation, leading to an effective survival fraction of 100% at 0 Gy dose.

For a given depth along the beam path in the water target we then plot the cell survival, corrected for the plating efficiency, vs. the dose for all measurements from a specific run. These data are then linearized and fitted to a double exponential of the form

$$\ln(SF) = -\alpha D - \beta D^2$$

From the fit results we extract the dose value required to achieve 10% survival (90% cell killing). Comparing this value to the SF_{10} dose of a reference radiation then allows plotting relative biological efficiency for each depth slice. Figure 3 shows an example of this analysis for the run time in 2010, comparing cell survival vs. dose for a slice near the distal edge of the Bragg peak (98 mm) and in the plateau region (50 mm) yielding a relative RBE of 1.5.

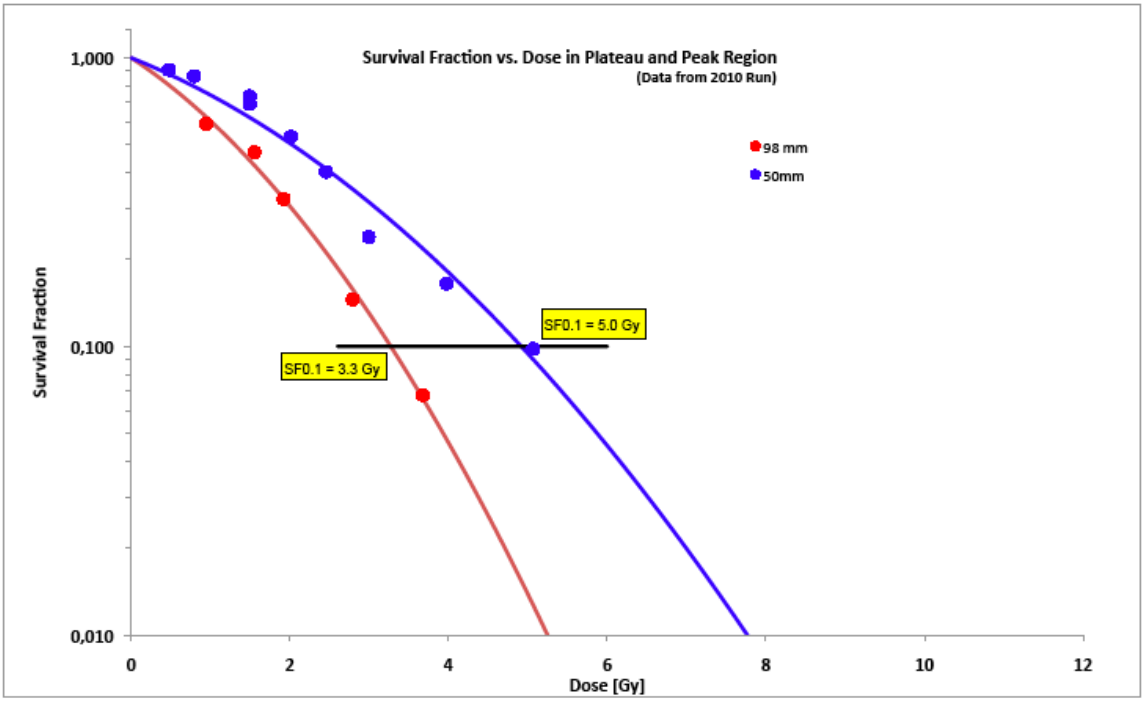

Figure 3: Examples of fits for plateau (blue) and peak (red) slices yielding a relative RBE of 1.5 comparing Bragg peak antiprotons to plateau antiprotons.

III. Meta analysis of all data sets

With the final data sets available now we have started the effort to combine all measurements in a consistent data set. Here a number of issues have to be addressed to allow the measurements of different years to be considered as "measurements under (nearly) identical conditions".

1. After 2009 we changed the physical setup, introducing the Mimotera silicon pixel detector as a shot-to-shot beam monitor. This introduced extra material in the beam path, shifting the distal edge of the Bragg peak in the water phantom forward, and possibly introducing additional lateral scattering and production of secondary particles.
2. During the course of the different experiments the FLUKA group released several upgrades, which led to a discrepancy between dose calculations and the benchmark measurements we performed in 2006. For a final analysis all dose calculations will have to be redone with an identical FLUKA release, preferably a well benchmarked one. This will require a further fine-tuning of the antiproton interaction cross sections for low energy antiprotons in the FLUKA data base, and we are currently discussing with the FLUKA team how to best achieve an adequate precision in the dose calculation of low energy antiproton beams stopping in a water target. With the limited beam time available in 2012 we were unable to generate a new set of depth dose curves for antiprotons and now plan to use the original measurements from 2006, as these were carefully compared to absolute dose measurements using Alanine, as well as relative dose measurements with a calibrated ionization chamber (Advanced Roos Chamber, PTW Freiburg, Germany). A detailed description of these benchmarking experiments can be found in references 14 and 15 in the Appendix.

   In order not to rely on a single MC particle transport code, efforts are also undertaken to benchmark and update the antiproton annihilation cross sections in the Russian code SHIELD-HIT currently maintained at Aarhus University. Related to this issue are choice and implementation of a proper model for annihilations of slow antiprotons on compounds, as simple Z-scaling may be insufficient here. Currently we have the impression that the cancellation of two errors eventually led to the excellent agreement observed in our 2006 benchmark. Correcting one of these errors after initial discussions with the FLUKA team therefore led to an inferior agreement. Rectifying this situation would not only enable us to obtain the final absolute dose values for all years, but also be beneficial to the community at large.
3. The shape of the physical dose distribution along the spread-out Bragg peak (SOBP) was not identical for all years – this will lead to a different mixture of low LET (in flight) and high LET (stopped) antiprotons in the proximal area of the SOBP (see figure 4).
4. Before 2010 beam profile monitoring could only be done by Gafchromic film. This often led to one film covering several of the shorter irradiations. Potential beam shifts or changes of the beam shape might have gone undetected in the averaging process of exposing film.

Points 3 and 4 will be difficult to correct for after the fact and will have to be considered as sources of systematic errors.

VI. Microdosimetric studies of antiprotons stopping in water.

Microdosimetry is the dosimetry on the length scale of micrometers. It offers a deeper insight into the biological effectiveness of different radiation types, as in this regime, the stochastical nature of energy deposition of the radiation becomes important and the length scales are comparable to typical size of cell nuclei. Large energy deposits within these small volumes, e.g. introduced by densely ionizing particles, are considered to be more biologically effective as many small energy deposits, e.g. by photons and electrons.

To supplement the clonogenic survival studies on V79 cells, in this study the microdosimetric properties of antiprotons stopping in water are investigated in terms of the lineal energy y. The lineal energy is the ratio of the energy ε imparted into a volume of interest by an ionizing particle and the mean chord length l within that volume: $y = \varepsilon/l$.

The detector used is a spherical tissue equivalent proportional counter (TEPC) with an outer diameter of 1.27 cm. It is filled with tissue equivalent gas with a low pressure such that the simulated diameter of the sensitive volume is approximately 2 µm. Lineal energy spectra were taken at different positions along the axis of a 126 MeV antiproton beam stopping in the water phantom of the AD-4 experiment: in the plateau (19.7 mm depth), in the Bragg peak (108.7 mm), as well as distal and lateral to the Bragg peak.

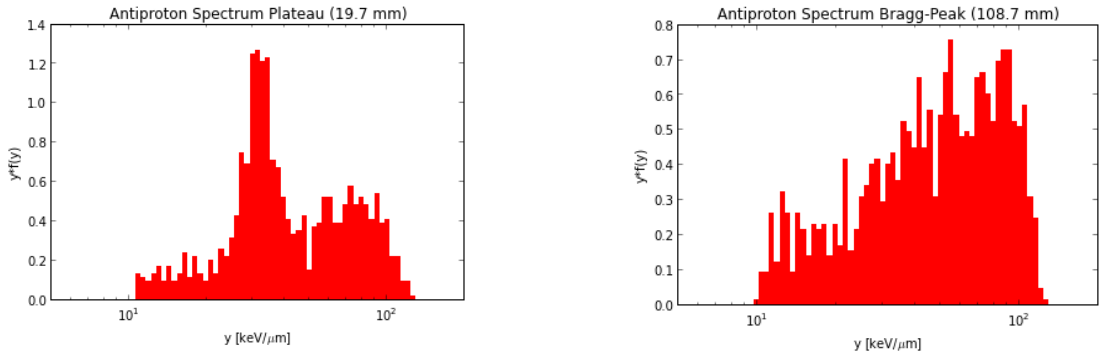

Figure 4 : Frequency distributions of lineal energy in the plateau (left) and in the Bragg peak (right) of a 126 MeV antiproton beam stopping in water.

In a first preliminary analysis, the frequency distributions of lineal energy of antiprotons are shown in Fig. 4. In the plateau (left) a pronounced component of medium lineal energies, possibly related to the stopping power of antiprotons, is visible, whereas in the Bragg peak (right), there is a broad spectrum of lineal energies, which is attributed to the highly mixed radiation field generated by the antiproton annihilations. The small peak at higher lineal energy seen in the left frame can be interpreted as the result of in flight annihilations of antiprotons.

However, the highly pulsed nature of the antiproton spill extraction present at the AD imposes challenges in the interpretation of the measured spectra as in theory, microdosimetric spectra are defined for single particle interactions. Thus, the large number of antiprotons per spill extracted may have introduced pile-up effects in the detector and its read-out electronics.

To compare the measured spectra to clinical proton and carbon ion beams, measurements at the Heidelberg Ion-Beam Therapy Center (HIT) are planned for the near future. To aid the understanding of possible pile-up effects for the antiproton spectra, Monte-Carlo simulations have been initiated. First results using the FLUKA transport code show that a simple sampling and summation of single events is not sufficient to fully reproduce the measured spectra. This probably must be attributed to restricted accuracy of the simulated results for very low-energy events.

V. Summary

Over the last years we have collected 5 independent data sets on survival vs. depth for 10% survival on V79 cells irradiated by antiprotons. As the physical parameters slightly differ from year to year (additional detectors placed in the beam, beam profile variations from year to year, possible changes in beam divergence, etc.) combining these data into a coherent data set is a tedious task. This work has been started, but not yet completed due to the uncertainties surrounding the dose calculation using FLUKA. Once this last point has been addressed a complete set of RBE values along the depth in water for a 126 MeV antiproton beam can be extracted from the total data set accumulated.

At that point we can expand the initial dose planning studies shown in earlier reports by including the actual variation of RBE with depth for both carbon ions and antiprotons, leading to a final publication on the RBE values for antiprotons and several examples of comparative dose planning studies for protons, carbon ions, and antiprotons. This work can then be the basis for decisions to continue the research on the possible use of antiprotons for cancer therapy in select cases either at the AD at CERN after the current shut down or at a future facility providing antiprotons at energies and intensities suitable for such research.

AD-4 has finished data taking under the original proposal. Our work has reached a closure of a chapter, but certainly not the book on antiproton cancer therapy. Any future work at CERN shall be based on a new proposal to the SPSC. For the benefit of our collaboration as well as of other current or future groups in need of an antiproton test beam, we strongly urge the AD community to keep the extraction capability at higher energies into the DEM line available.

List of publications from the AD-4 Collaboration

1. JN Kavanagh, FJ Currell, DJ Timson, KI Savage, DJ Richard, SJ McMahon, O Hartley, GAP Cirrone, F Romano, KM Prise, N Bassler, MH Holzscheiter, G Schettino; Antiproton induced DNA damage: proton like in flight, carbon-ion like near rest; submitted to *Nature Communication*.
2. Stefan Sellner , Rebecca Boll, Massimo Caccia, Loretta Negrini, Tina Straße, Sara Tegami, Michael H. Holzscheiter, and the ACE collaboration; The antiproton cell experiment – do antiprotons offer advantages over other particle beam modalities?; *Hyperfine Interactions* (2012) 213, 159-174
3. Stefan Sellner, Carsten P. Welsch, Michael Holzscheiter; Real-time imaging of antiprotons stopping in biological targets – Novel uses of solid state detectors; *Radiation Measurements* 46 (2011) 1770-1772
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12. Niels Bassler, Jan Alsner, Gerd Beyer, John J. DeMarco, Michael Doser, Dragan Hajdukovic, Oliver Hartley, Keisuke S. Iwamoto, Oliver Jäkel, Helge V. Knudsen, Sandra Kovacevic, Søren Pape Møller, Jens Overgaard, Jørgen B. Petersen, Timothy D. Solberg, Brita S. Sørensen, Sanja Vranjes, Bradly G. Wouters, Michael H. Holzscheiter; 'Antiproton radiotherapy', *Radiotherapy and Oncology* 86: pp. 14-19 (2008)

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PhD Theses:

Sara Tegami
LET measurements with a Liquid Ionization Chamber; Max Planck Institute for Nuclear Physics & University of Heidelberg (2013)

Joy Kavanagh
DNA damage and biological effectiveness of antiprotons in relation to carbon-ions and protons; Centre for Cancer Research and Cell Biology School of Medicine, Dentistry and Biomedical Sciences Queen's University, Belfast (2012)

Rochus Herrmann
Prediction of the Response Behaviour of One-Hit Detectors in Particle Beams; Department of Physics and Astronomy, Faculty of Sciences and Technology, Aarhus University (2012)

Roy Keyes
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Loretta Negrini:
The MIMOTERA: a silicon pixel detector for beam profilometry; University of Insurbia, Como, Italy (2012)

Tina Straße
Generation of a Spatially Extended Bragg Peak to Investigate the Relative Biological Effectiveness (RBE) of Antiprotons; Max Planck Institute for Nuclear Physics; University of Heidelberg (2011)

Matteo Maspero
Beam profilometry and solar physics experiments with an ultra-fast monolithic active pixel detector; University of Insurbia, Como, Italy (Diploma 2012)

Rebecca Boll
A Monolithic Active Pixel Sensor as Direct Monitor for Therapeutic Antiproton and Ion Beams; Max Planck Institute for Nuclear Physics; University of Heidelberg (2010)

Loretta Negrini
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Stefan Sellner
Real-Time Imaging of the Annihilation Vertex Distributions for Antiprotons Stopping in Biological Targets; Max Planck Institute for Nuclear Physics; University of Heidelberg (2010)