Report on Experiment AD-4/ACE

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I. Introduction:

One of the predominant goals of particle beam cancer therapy is to achieve the best ratio of dose delivered to the targeted tumor volume to the unwanted dose to surrounding healthy tissue and critical organs. This can be obtained using protons or heavier ions due to the inverse dose profile described by Bethe's formula for the stopping power for charged particles travelling in matter. Charged particles have a finite range in matter and deposit the highest dose near the end of range, forming the Bragg peak, which is in strong contrast to the exponential loss of energy for X-rays (Figure 1). This physical property of heavy charged particles has led to the development of proton and carbon ion beam therapy with an increasing number of clinical centers treating patients world wide.

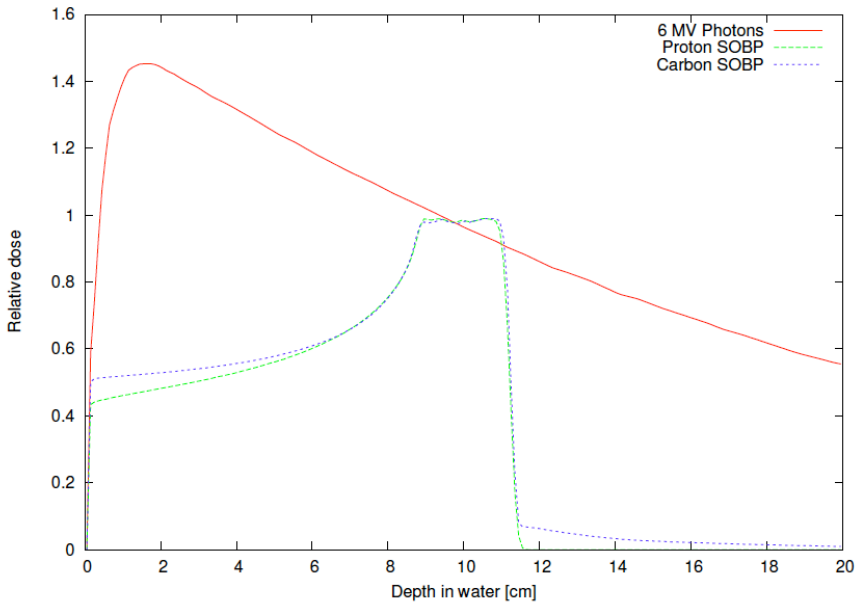

Fig. 1: Comparison of physical dose distribution vs. depth in water for X-rays, protons, and carbon ions (physical dose only).

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From the very beginning physicists and biologists have searched for further enhancements of the advantages provided by the physical behavior of charged particles passing through matter. Biological research showed early on that particles heavier than protons deliver a higher impact on the tumor even for an identical energy deposition (physical dose) to the target. This has led to the definition of the "relative biological efficiency" (RBE) describing the enhanced effect on living organisms due to the higher ionization density of heavy charged particles compared to X-rays and protons.

$$RBE = \frac{Dose_{reference}}{Dose_{test\_radiation}}\Big|_{iso\_effect}$$

At the same time physicists continued to study more exotic particles to achieve a modification of the physical dose profile, resulting in an intensive effort to use pion beams, which, due to their annihilation, would provide additional dose deposition near the end of range. Clinical programs were initiated at Los Alamos National Laboratory in the United States, Paul Scherrer Institut in Switzerland, and at TRIUMF in Vancouver, Canada. Due to poor dose confirmation caused by the extent of the annihilation "stars" and the beam contamination with muons and electrons, amongst other reasons, these were eventually abandoned.

Antiprotons were first proposed as therapeutic particles by L. Gray and T. E. Kalogeropoulos in the early 1980's [1,2]. In their 1989 paper T. E. Kalogeropoulos and R. Muratore [3] showed that for a given dose to a tumor volume antiprotons would deposit less than half the dose to tissue outside the target than other particles like protons, Carbon ions, or pions. Initial experimental tests were performed by A. H. Sullivan [4] who confirmed a factor of two enhancement in physical dose in the peak for identical dose at the target surface, but unfortunately he put this in relationship to the 2 GeV annihilation energy and deemed it as irrelevant. This led to a delay in experimental work until the early 2000's when the AD-4 experiment was proposed by the ACE collaboration and approved for running at the AD at CERN by the Research Board.

The goal of the AD-4/ACE collaboration is to place the discussion of the therapeutic advantage of antiprotons over other particles on a firm experimental basis, including the biological enhancement that has been expected due to the heavy secondary particles resulting from the annihilation at rest in the target. At the time of approval of AD-4 the source of low energy antiprotons at CERN had been non-negligibly changed compared to the original LEAR (Low Energy Antiproton Ring). This had a significant impact on our capabilities to perform these studies. Instead of a continuous beam of antiprotons of around 10^6 antiprotons/second antiprotons were now only available in 500 ns pulses with approximately 3 x 10^7 antiprotons at time intervals of around 90 seconds, making dosimetry and beam monitoring a non-trivial task, and extending the time duration for a single experiment to a length that raised concerns about effects on survival and repair of cells during the course of a single experiment.

After approval of the experiment a first set of studies was performed using a beam of antiprotons extracted from the Antiproton Decelerator (AD) at 46.7 MeV kinetic energy, as this was possible without effecting the other experiments in a significant way. It allowed performing several short experiments of duration between 1 and 2 days over the two year period of 2003 and 2004, demonstrating the significant enhancement in cell killing capability of antiprotons compared to protons by nearly a factor of 4. As no absolute dosimetry was available to us at that time, we used the Biological Effective Dose Ratio (BEDR), the ratio of cell killing in the peak to the entrance channel (plateau), as a surrogate figure of merit instead of measuring RBE. The major drawback of this method was in the fact that this number is dependent of the specific experimental set-up and cannot be compared with other modalities presented in the literature if the experiments were not performed in the exact same way. In addition, at the relatively low kinetic energy of 50 MeV the penetration depth in the target (accounting for additional material in the beam for beam monitoring and containment vessels) was only around 15 mm, making a differentiation between peak and plateau very difficult.

Based on the initial success of these measurements the collaboration decided to push forward with an extended proposal, asking CERN to provide a special beam at kinetic energy of around 126 MeV, which was not only deliverable by the AD with minor modification of operation, but also provided a penetration depth in the target of beyond 10 cm, providing a clear separation of peak and plateau and enabling us to form a clinically relevant Spread-Out Bragg Peak (SOBP) of 12 mm depth. An example of a typical depth dose distribution used from there on is shown in Fig. 2.

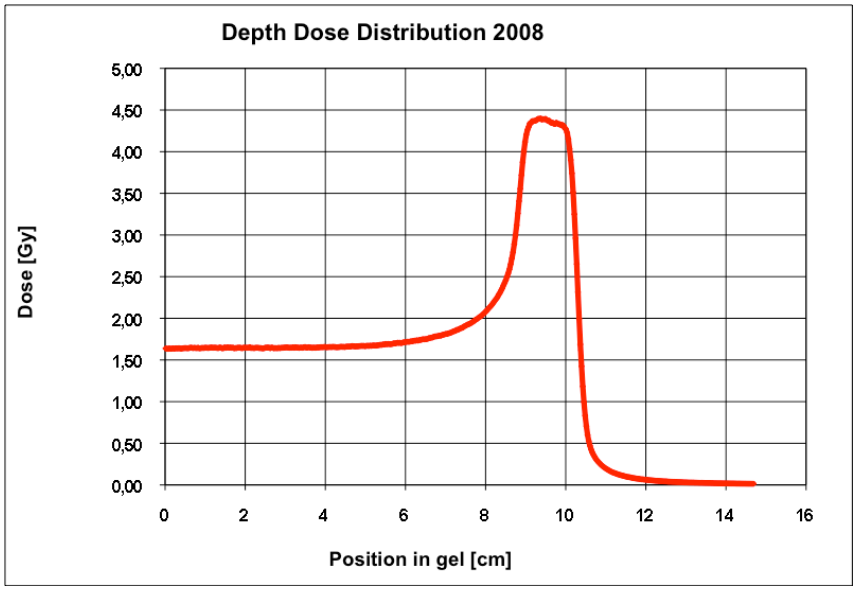


Fig. 2: Typical depth dose distribution delivered to the 6 mm diameter target tube in the AD-4 Experiment. The diameter of the beam was chosen with a FWHM of approximately 1 cm to assure a reasonable flat radial coverage of the target while at the same time maximizing the beam usage.

The first phase of the experiment at 46.7 MeV has been published in detail [5] and this report will solely concentrate on the measurements at 126 MeV between 2006 and today. Due to the different operation modus required from the AD at this energy it was no longer possible to quickly switch back and forth between normal operation and our experiment, which led to an agreement that AD-4 would be provided one full week of beam at 126 MeV each year. Each year was thus providing one complete data set for the biological effectiveness of antiprotons and additional beam time for dosimetry and auxiliary biological studies relevant to the question of the therapeutic potential of antiprotons (with the exception of 2012 where we were able to organize two independent experiments to measure RBE under identical conditions during the one week period).

In the following we will describe the experimental set-up, the methods and materials used, the data collected, and the current status of data analysis.

II. The AD-4/ACE Experimental Set-Up

The antiproton beam is exiting the vacuum of the DEM line through a 25 micron titanium window into air and then passes through a variety of beam monitoring and beam energy modifying layers.

The first is a wheel with PMMA slabs with thicknesses of 0, 2, 4, 6, 8, 10, and 12 mm that can be rotated into the beam path remotely via computer control. Positive feedback is provided through 3 digit binary codes on the individual slabs, read out via infrared diodes/sensors to assure proper positioning of this system at all times. Each of the PMMA thicknesses modifies the energy of the beam by a small amount and leads to a pristine Bragg peak at a slightly shifted depth. Overlaying carefully weighted fluences of the different energy beams thus allows construction of the prescribed spread-out Bragg Peak (SOBP).

Since 2009, following the degrader section a pixilated silicon detector (MIMOTERA) is mounted that is capable of monitoring the beam profile and position shot-by-shot. This turned out to be a crucial addition as sudden changes in beam shape and position could be detected immediately and data collection could be halted until the problem was resolved. In addition to online monitoring of the beam the data from the Mimotera detector can also be used as supplemental information in the determination of the beam conditions during irradiation (see section III.b).

After the degrader and beam monitor sections the beam traverses a calibrated ionization chamber[1]. The measured charge produced in the active volume of this chamber can be directly related to the total dose deposited in the chamber and constitutes an important input in the calculation of the depth dose distribution in the target volume.

Following the ROOS chamber a sheet of Gafchromic®EBT dosimetry[2] film is inserted for each individual experiment. From this we can deduce a precise profile

[1] *Advanced ROOS® Electron Chamber, PTW –Freiburg, 79115 Freiburg Germany*

[2] *Harpell Associates Inc. Oakville, Ontario, Canada L6L 2X4*

of the beam at the film position, which is the primary input to the beam definition for our Monte Carlo calculations of the dose delivered to the target.

After passing through the film and a 3 mm thick PMMA window the beam finally enters the phantom, which contains the gelatin/cell tubes.

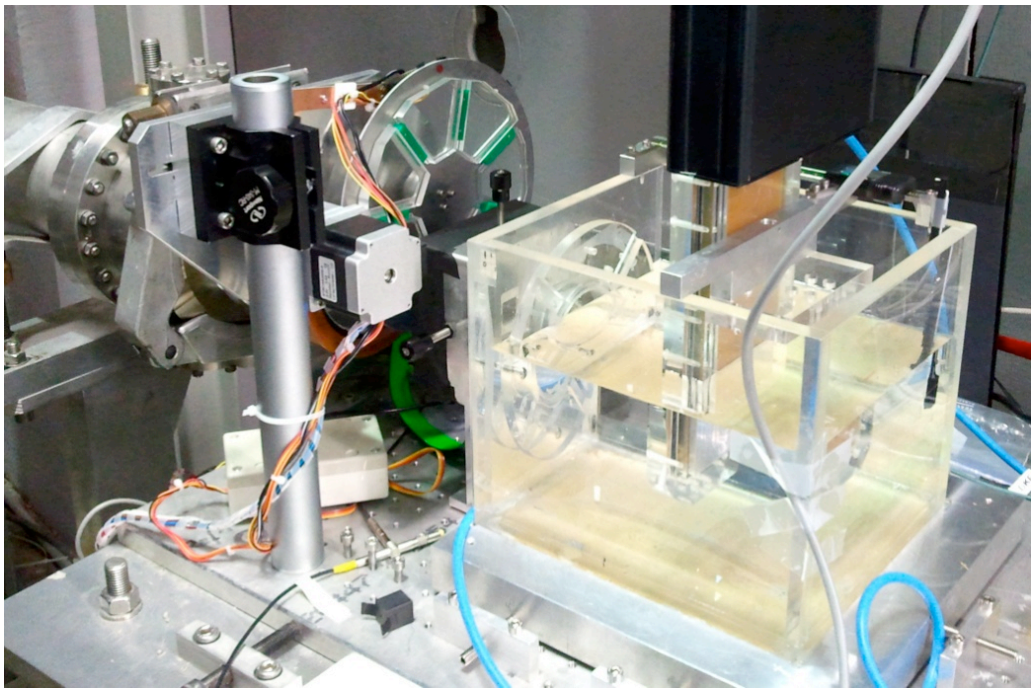


Fig. 3: ACE set-up at the DEM beam line. Visible are from left to right the end of the DEM beam line, the degrader wheel, the holder for the MIMOTERA detector and the phantom with a liquid filled ionization chamber installed to study LET distributions of antiprotons stopping in water. The ROOS chamber is mounted directly before the entrance window of the phantom (not visible).

III. Material & Methods

(a) Biology

The target of antiproton irradiation in AD-4 are V79-WRNE cells derived from Chinese Hamster lung fibroblasts, which is a cell line often used for radiobiological studies. The usage of a commonly used cell line, of which a large stock is stored frozen, allows different experiments conducted in subsequent years to be combined into one data set and also to compare our results to other radiobiological experiments with different ions published in the literature.

All cell preparation and analysis work is conducted in the radiobiological laboratories of the Experimental Clinical Radiation Oncology group of J. Overgaard at the Aarhus University Hospital in Aarhus Denmark. Cell preparation starts approximately 14 days prior to the experiment when cells are taken out of the -80°C

freezer and placed in cell medium at 37°C. This allows the cells to grow and divide, so that a high number of cells are reached for the experiment.

Immediately prior to cell samples leaving the laboratory in Denmark cells in the log phase, where the majority of the cells are rapidly dividing and are therefore randomly distributed throughout the phases of the cell cycle, are harvested, placed into a gelatin mixture and then injected into tubes. Cell tubes are cooled to 4 °C until the gel is solidified. Tubes are kept on ice, at or below 4°C, during transport from Aarhus to CERN and back as well as during the entire experimental period at CERN. This inhibits cell division and the cell repair mechanism that could distort the outcome of the experiments.

The gelatin mixture consists of 12% agar gel, 20% serum, and cells in growth media. To assure that the density of the cell/gel mixture matches the specific density of surrounding tube walls to avoid artifacts caused by differences in particle range in the different materials in radial direction, a master mix of 24% gel in cell medium is prepared, and the density is measured in each new batch by weighing a known volume with an accuracy of +/- 0.003g/ml.

On the day of the scheduled experiment cells are flown from Aarhus to Geneva. Upon arrival individual tubes are mounted into the phantom and irradiated at prescribed doses that constitutes best guesses for the dose levels to achieve cell survival between 100% and 1%. The phantom (PMMA tank filled with a mixture of glycerin and water to match the density of the gelatine/cell mixture and the sample tubes) is kept at 4° C to inhibit repair mechanism during long irradiation periods, and to prevent cells from dividing until they are seeded out in appropriate cell numbers (this is so we can monitor the number of cell divisions – we score a colony containing ≥50 cells as a surviving cell, which equals at least 6 cell divisions).

At the end of a specific experimental section (for which the time between harvest and fixing is kept to less than 48 hours, which has been tested to not influence cell survival) the gelatin/cell mixture is extruded from the tubes and sliced into 2 mm slices, with each slice containing approximately 140.000 cells. Selected slice positions are placed in 1ml growth medium at 37°C, which melts the agar. The agar/medium mixture is so dilute, that the agar does not interfere with any downstream applications. Cells are seeded out in a six-well plate, with 4 replicates for each sample. The number of cells plated in each well is chosen for optimal number of colonies (70) for the estimated dose delivered (exact doses delivered are not yet known at this point), with the minimum cell number 128 (for the control samples) and up to 20.000 cells, which is the maximum number of cells that can be added per well. More cells per well would be causing the high density of cells to interfere with the survival fraction. To ensure exact numbers of cells in each well, since 2008 the cells are seeded out using a Fluorescence Activated Cell Sorter (FACS), which counts each individual cell. Control tubes with non-irradiated cells are included for the whole procedure, including the journey to and from CERN. The fraction of colony forming cells in these control tubes enables calculation of the Plating Efficiency (PE) for the cells, used to correct the experimentally determined survival fractions for any non-irradiation related cell deaths.

After 12-14 days surviving, i.e. proliferating, cells have divided at least 6 times, resulting in colonies of ≥50 cells. Cells are then fixed and stained, images of the well

plates are scanned by a flat bed scanner, and the number of colonies in each well is determined by computer with freely available software (ClonoCounter). The software is recognizing colonies based on parameters for threshold grey level; minimal size of a single colony, and grey width (the tone of grey, which enables the software to recognize single colonies from clustered colonies). Previous correlation analysis using ClonoCounter has revealed a highly positive correlation between the absolute number of colonies counted manually and automated.

At this point plots of the survival fraction (SF) defined as:

$$SF = \frac{Colonies\_counted}{Cells\_seeded} \times PE$$

vs. depth can be generated. Figure 4 shows a sample of a survival vs. depth curves in the target for the years 2008 and 2010 for a variety of target (plateau) doses.

Despite following a seemingly straightforward standard procedure used in many laboratories worldwide, the special circumstances under which AD-4 operates are the cause for some concerns.

(i) Unlike in radiobiological measurements using well characterized and calibrated clinical radiation units the radiation dose at the AD-4 is not well known a priori. With survival being a strong function of dose this can lead to over- and under-dosage if dose estimates turn out to be erroneous and potentially can cause loss of data points. To predict a specific survival level we assume a beam profile and beam divergence as input to a Monte Carlo calculation using the FLUKA code package together with experiences in survival vs. dose from previous years to arrive at a prescribed number of antiprotons. These calculations are time intensive and when at the beginning of an experiment it becomes apparent that beam profiles are different from the assumed values new FLUKA calculations are often not possible and quick adjustments are necessary.

(ii) Because of the relatively narrow width of the beam a variation in beam intensity across the cross section of the tube exists and while we aim at less than 10% intensity variation over the diameter of the tube, this could be significantly larger and varying with depth in the target if the beam axis and the tube axis are not properly aligned. We have the mechanical alignment of the set-up carefully checked prior to each run by professional surveyors from the CERN technical support, but the degree of (mis)alignment of the beam with the axis of the vacuum tube is not well known. To minimize the impact of alignment errors, we initially install the target tank without filling it with water so the beam penetrates both front and back walls. Placing sheets of EBT film in front and on the back of the tank allows us to determine the overlap of the beam center with the center of the tank to within 1mm or less. But once the experiments start and the tank is filled with liquid and the target tubes are installed we can only monitor the relative position of beam and tank center at the entrance side (again to approximately 1 mm accuracy), but possible changes of either the beam direction or the tank positioning are no longer detectable.

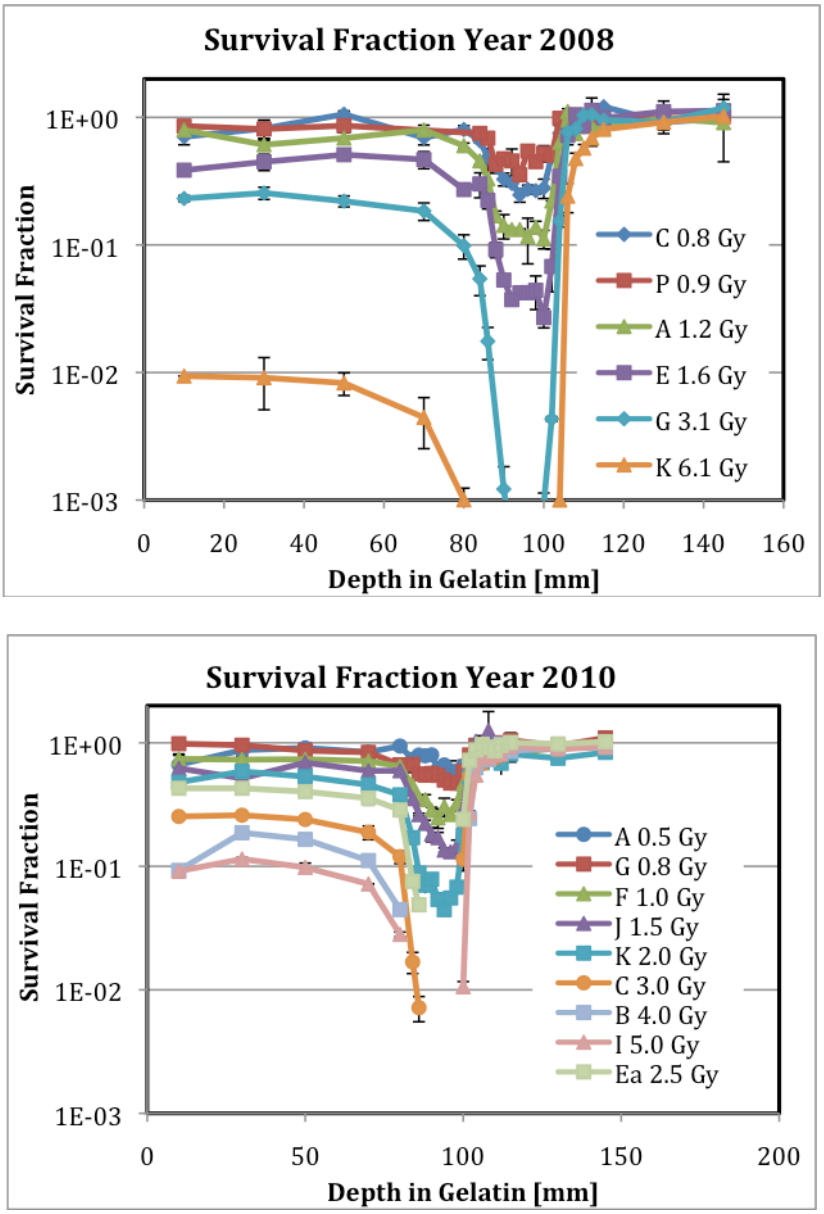


Fig. 4: Survival curves for different values of plateau dose obtained in the years 2008 and 2010. Labels are prescribed doses only and do not necessarily constitute the exact dose delivered in the actual experiment.

(iii) Aside from dose estimation and alignment errors, biology itself can also cause systematic errors in the final results. To separate effects on cell survival due to the irradiation from other causes we carry a set of control tubes through the entire procedure and analyze these at the end of the experiment with exactly the same procedure. The ratio of counted cells to seeded cells in these tubes is used as the so-called "plating efficiency" and is assumed (and has been experimentally confirmed) to be essentially constant over the course of the 48 hours used for a single experiment. Nevertheless, in a few cases we have observed that this

determination of the plating efficiency, due to the biological variance that influence all cell experiments, can lead to cell survival results in the low dose regime of larger than 100%, which obviously is incorrect. Therefore we have also prepared for a second method to determine plating efficiency using the uncorrected cell survival data plotted against the actually applied dose. These data are then fitted to the standard double exponential description of cell survival using the cell survival at zero dose as a free parameter:

$$SF(D) = SF(D=0) \times \exp[-\alpha D - \beta D^2]$$

While the accuracy of this determination of the Plating Efficiency (PE = $SF_{D=0}$) is strongly limited by the scarcity and scatter of data points obtained from an individual slice in a given target tube, it could help confirm the assumption of constant PE over the course of an experimental run. As this method needs the exact dose delivered at any depth point used, this is only possible retrospectively after the experiment, when the final dose calculations based on the beam monitoring during the run have been completed.

(b) Physics

As mentioned in the introduction, aside from the enhanced physical dose of antiprotons the main parameter in question is the biological enhancement of antiproton beams compared to standard x-ray or proton beams, i.e. the RBE of antiprotons. The ultimate goal of AD-4 is to provide a database of the biological effective dose of an antiproton beam stopping in water throughout the entire depth dose distribution, that can then be imported into clinical dose planning platforms in order to compare dose plans for specific tumor sites using antiprotons, protons, and carbon ions.

To obtain the RBE from cell survival data at any given depth along the beam path up to the Bragg peak requires exact knowledge of the depth distribution of the physical dose delivered. In AD-4 this is done by careful online beam monitoring during the run followed by Monte Carlo calculation after completion of the run.

The two critical inputs in this process are the correct description of the beam geometry and the experimental set-up and the correct handling of all interactions of antiprotons with biological target media by the Monte Carlo code in the relevant energy range.

For the description of the beam we ideally need to know the size and position of the beam source and the divergence of the beam at the source. Then we can transport the beam particles with Monte Carlo models through the different materials into the biological target up to the end of range.

As the beam source in the DEM line is not exactly known, we use the beam profile measured at the entrance to the phantom using GAFchromic films and then pick an arbitrary, but reasonable, combination of source distance and beam divergence to generate the observed beam profile at the film location. GAFchromic

films are a standard tool for radio-oncology beam diagnostics and quality assurance. The film darkens upon exposure to ionizing radiation and individual film batches can be calibrated using standard irradiation equipment. A linear response in dose is seen over a wide range of doses and a fit to the density profile of the film has been observed to give a good description of the beam spot. We use pieces of film at the very entrance to the phantom for each individual experiment (or several films if the experiment may take a long time). After irradiation the film is scanned on a flatbed scanner and the density of the film is digitized using the standard image software package ImageJ, developed by the National Institute of Health (NIH) [6]. We take a cut along the vertical or horizontal axis (or, in case of an elliptical beam profile, along the minor and major axis) and fit the data along these lines to a Gaussian. Using markings on the film made when the film was mounted to the tank we determine the center of the beam. Data for beam profile, rotation (of non-circular beam spots), and center of beam spots are used as input parameters for an iterative process to determine the beam spot size and the beam divergence in the source plane, using Monte Carlo calculations.

In addition to the images obtained on the Gafchromic film, we can use our pixel detector mounted about 10.5 cm before the Gafchromic film and compare the profiles measured in the MIMOTERA and the EBT film. The beam diameter on the EBT film will be slightly larger due to the scattering in the pixel detector and the a priori unknown beam divergence. This can provide additional information on the actual beam divergence. As an alternate approach, at one time, we used the MIMOTERA detector to measure the beam profile at different positions along the beam axis of our experimental area before mounting any of the other components. These studies are currently being analyzed and the final results should be available soon.

To estimate the potential impact of such geometrical uncertainties on the final analysis of the biological data sets we have accumulated due to remaining uncertainties in beam definition and geometry, we performed a number of Monte Carlo simulations using a multitude of possible values for the critical input parameters. One example is shown in Figure 5, where we used different source distances and adjusted the combinations of source size and divergence to regenerate the beam spot observed on the EBT film.

The variation in peak dose for identical plateau dose is of the order of 4 % which needs to be added to the overall error analysis, if it cannot be improved through the aforementioned analysis.

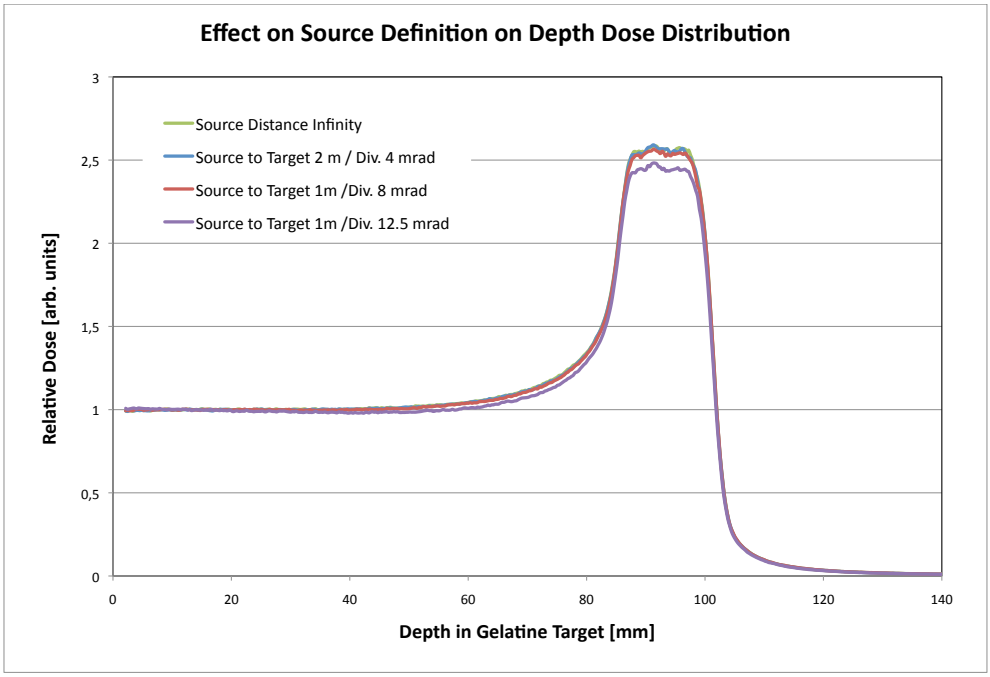


Fig. 5: Dose profile of SOBP calculated with FLUKA using different definitions of the beam, all generating the same beam spot in the plane of the EBT-2 Film

(c) Computational issues

For these calculations and for all Monte Carlo support of the planning and data analysis we use the Monte Carlo package FLUKA [7,8]. Unlike GEANT, FLUKA is a closed code, which represents the best possible description of the known physics available to the FLUKA development team at the time of release for a specific version. Updated versions have been released over the course of the years relevant to our experiment as part of the constant pursuit of the FLUKA team to improve the physics description of all relevant processes over a wide range of energies.

As we observed that different FLUKA versions produced different depth dose distributions for our experimental set-up using identical input parameters we initiated a scientific exchange with the FLUKA team and scheduled a detailed discussion of the relevant questions with A. Ferrari from the FLUKA Team at our annual workshop in the summer of 2013. As an outcome of these discussions our understanding of the FLUKA code was significantly enhanced and in addition to the current version available online (2011.2b) we also were given access to the latest developmental version that is believed to best describe the low energy interaction of antiprotons with matter (FLUKA_DEV). In addition, it was suggested to us by A.

Ferrari to change some of the default values for specific cards describing the capture probability for antiprotons in high Z atoms (FLUKA_DEV_LIM).

Several test calculations have been performed and can be grouped into two categories: (a) using different versions of FLUKA for identical input files to study the effect of program updates over the period of the AD-4 experiment, and (b) varying input parameters describing the experimental set-up (i.e. beam definition) to study the sensitivity of the final outcome of the experimental analysis to the definition of the geometry.

Figure 6 gives a few examples for case (a) where we recalculated specific analyses from earlier years using the original geometry input to FLUKA. Shown are a recalculation of our original benchmark experiment published in 2008 [5], a pristine peak from the 2008 experiment calculated with FLUKA 2008 v.3b.0 and the FLUKA 2013 beta version, and a recalculation of the depth dose distribution of the spread-out Bragg peak from the 2010 experiment using FLUKA 2008 v.3b.1 compared to the latest published version. From these examples it becomes clear that after a final decision has been reached which version and thresholds to use all analyses from prior year have to be re-run.

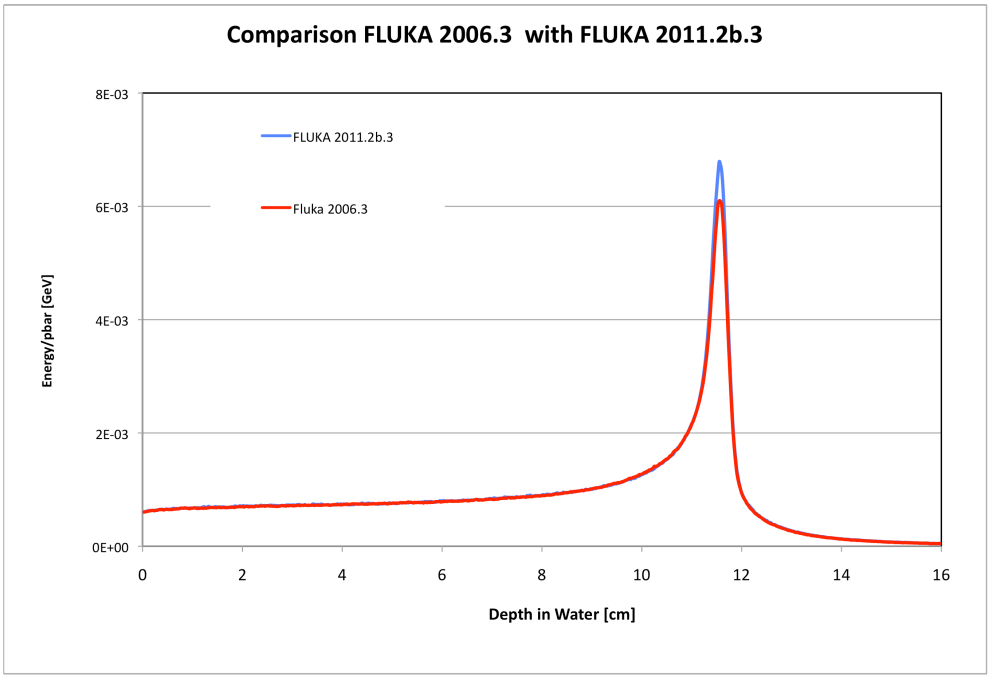
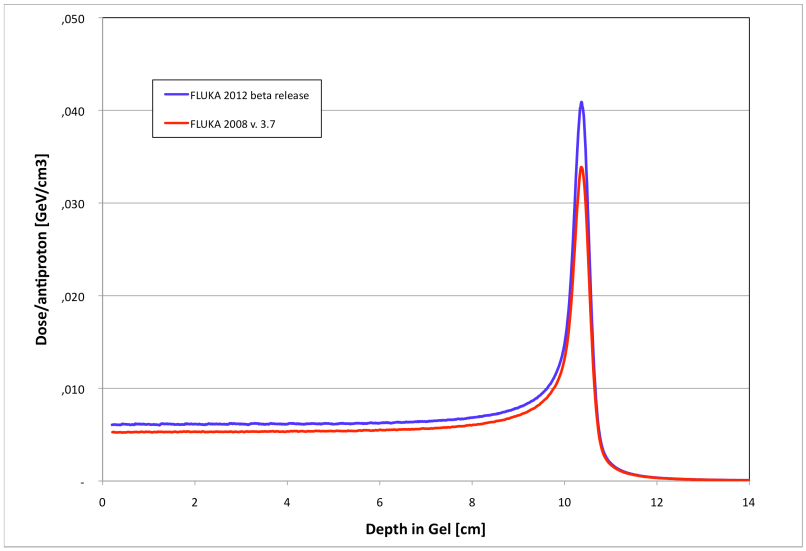


Fig. 6a: Calculated dose profile for the 2006 benchmark experiment described in [5], using both the original FLUKA release at the time (FLUKA 2006.3) and the currently available standard version 2011.2b



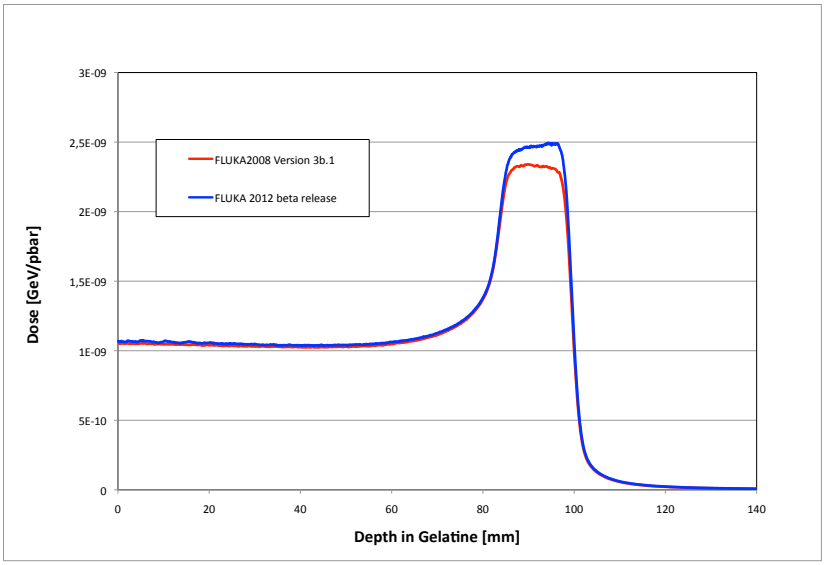


Fig 6b.: Effect on depth dose calculations by the choice of FLUKA version. Upper frame: calculation of pristine Bragg Peak in water using the 2008 geometry input file and both the 2008 FLUKA release and the 2013 FLUKA beta release. Lower frame: Effect on shape of SOBP due to the changed peak-to-plateau ratio resulting from the 2008 and 2013 FLUKA release.

To aid the decision on the choice of version suited best for our experiment we have used a part of the 2012 beam time to perform another set of benchmark experiments, irradiating stacks of Alanine pellets. L-α-Alanine is an amino acid that occurs naturally in the human body. When Alanine is irradiated with ionizing radiation, it forms the stable radical $CH_3 - \dot{C}H - COOH$. Using an electron spin resonance (ESR) reader, the free electron pair at the chiral carbon atom can be detected. The magnitude of the ESR response depends on the amount of absorbed dose. The dynamic range of these pellets is large, ranging from 0.5 Gy to 100 kGy, being linear in the region up to 10 kGy. The response of Alanine detectors to heavy charged particles was investigated by Hansen and Olsen in the 80s who developed a model explaining the behavior based upon the Butts and Katz track structure [9]. More details on this can be found in our paper on the 2006 benchmark experiment with Alanine [10].

Aside from irradiating Alanine pellets situated in the plateau region, as we have done in all prior years to provide an absolute calibration of our dose estimates, we also build an extended stack as shown in figure 7 to measure the absorbed dose in both the plateau region and a spread-out Bragg peak produced with our degrading wheel, knowing that the largest deviation between different releases of FLUKA occurred in the peak.

The Alanine pellets and the read-out were provided to us free of charge by Hugo Palmans and Peter Sharpe from the National Physics Laboratory (NPL) in the UK.

In Figure 8 we show the dose response values measured with the Alanine pellets in comparison to the calculated dose using the current standard FLUKA release, corrected for the Alanine response using the Hansen-Olsen Model.

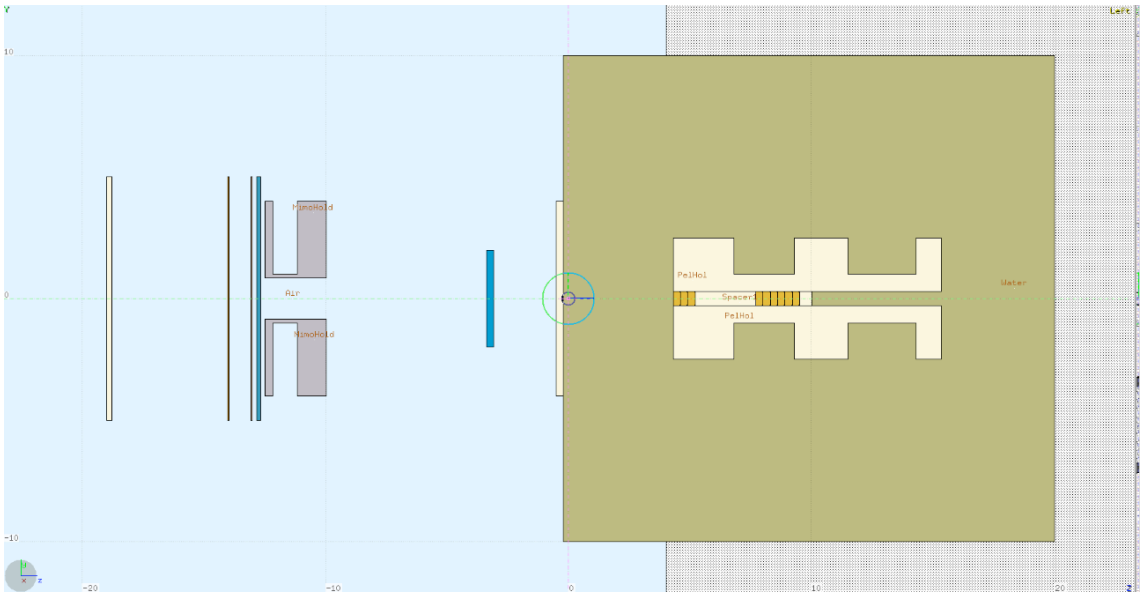


Fig. 7: A graphical rendition of the input file used to model the geometry of the test irradiation of the SOBP Alanine stack during the 2012 beam time.

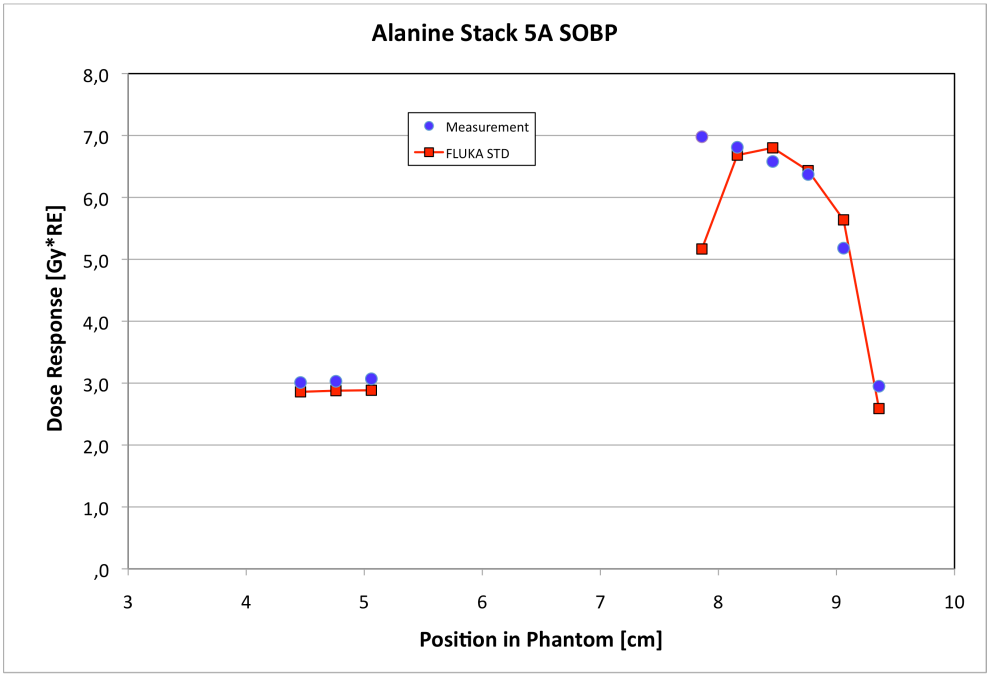


Fig. 8: Comparison of Alanine measurements in a spread-out Bragg peak with Monte Carlo calculations using FLUKA 2011.2b.3.

The agreement between measurements and calculations is better than 6% for all data points with the exception of the last point, where a slight axial displacement of the Alanine stack together with the steep drop-off of the dose at the distal edge could dramatically effect the accuracy of the method, and the very first point in the SOBP region, which lies significantly higher than the measured value. The reasons for the discrepancy of this particular data point are the topic of ongoing studies and discussions within the collaboration.

Defining the figure of merit Δ as

$$\Delta = \frac{1}{n}\sqrt{\sum_{i=1}^{n}(X_{i,m} - X_i)^2 / X_{i,m}^2} \ ,$$

using $X_{i,m}$ for the measured values and X_i for the values calculated with the three FLUKA versions available to us, we arrive at $\Delta = 2.3\%$, 6.0%, and 7.5%, for the FLUKA versions 2011.2b, DEV, and DEV_LIM respectively.

Figure 9 gives a graphical representation of the differences between the different FLUKA versions and provides a visual confirmation of the above analysis. All FLUKA versions agree reasonably well in the plateau region, the standard version and the developmental version produce results very close to each other also in the Bragg peak, but the developmental version with modified defaults shows a significant over estimate of dose in the latter half of the SOBP.

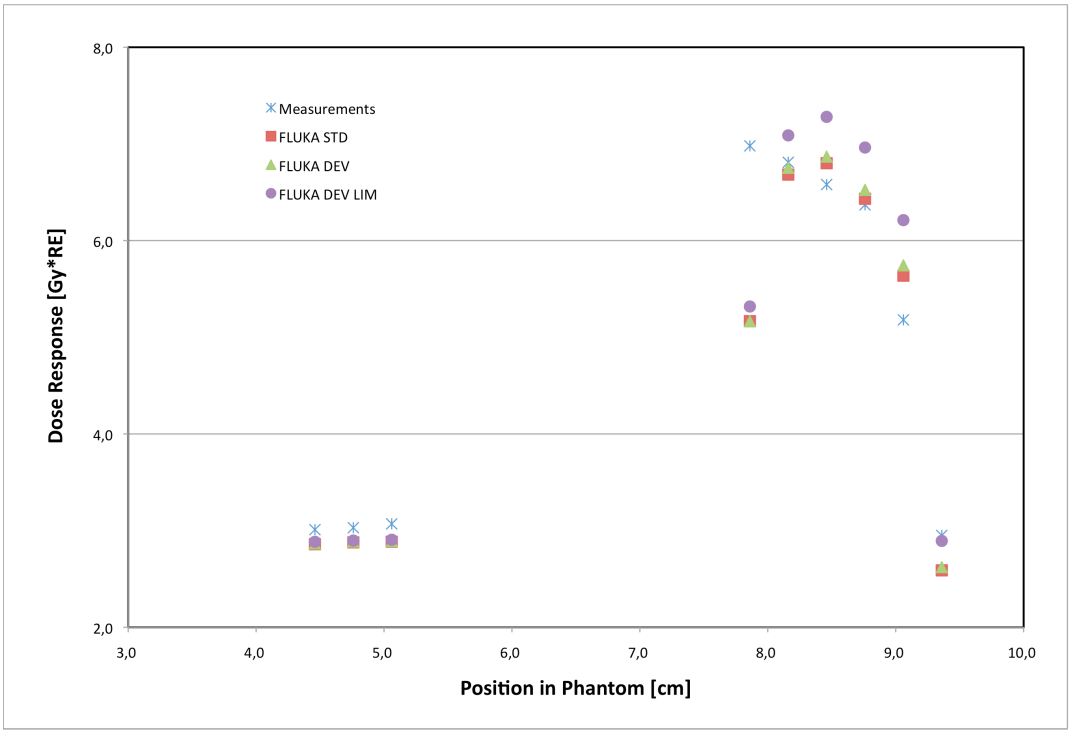


Fig. 9: Results from calculations using three different versions of FLUKA available to us: the current official version available on the FLUKA web site (red squares); the developmental version provided to us by the FLUKA team (green triangles); and the developmental version with modified defaults (purple dots).

We are currently investigating possible explanations for the strong deviation between measurement and calculations of point 4, ranging from read-out problems, to geometry errors, to labeling errors for the specific pellets and look for possible refinements of our analysis. Unless these efforts result in a completely different verdict for the agreement between calculations and measurements we will use the standard version of FLUKA on all input files from the years 2008 – 2012 to calculate the final doses delivered to the samples. This will then allow us to combine all data

into a single data set, resulting in a functional dependency of RBE vs. depth that can be compared to measured RBE vs. depth profiles for carbon ions.

IV. Summary and Conclusions:

Following initial successful measurements of the biological effective dose ratio (peak to plateau) at low antiproton energy, the AD-4 collaboration has started in 2006 to perform one week of experiments per year at the AD facility at a special beam setting providing 126 MeV kinetic energy (502 MeV/c momentum). Each year a number of days was used for dosimetric studies, detector development, and beam developments, with the rest of the time fully dedicated to radiobiological studies. Latter were concentrating on the measurement of cell survival along the depth dose profile of an antiproton beam entering a biological target and stopping at approximately 10 cm depth in the target medium. Some alternate experiments studying the damage and repair response of cells irradiated directly by antiprotons or as a result of cell-to-cell signaling were also performed and have been published separately [11,12].

Over the time between 2008 up to 2012 we have collected 4 complete sets of data from independent experiments under similar conditions. Some corrections for minor changes in the geometrical set-up need to be applied, but are well understood, to combine the data into one set. In addition we have collected partial data sets in 2007, 2009, and 2011 that can be incorporated into the final analysis.

The experimental analysis consists of two arms (a) biological analysis of cell survival at various depth points in the target medium, corrected for cell loss due to a plating efficiency less than 100%, and (b) determination of actual dose delivered to any depth point in the sample during irradiation.

Part (a) is essentially completed and the data are awaiting the results from the dose determination effort. Here we combine accurate monitoring of beam intensity and beam shape with knowledge of the physical set-up and use the Monte Carlo code FLUKA to determine the final dose values. As it was observed that new releases of FLUKA led to differences in dose for identical input files, we conducted a number of benchmark measurements that can help in deciding on which of the three different versions currently offered to us by the FLUKA team are best suited for our situation. At the current status of this analysis the collaboration is of the opinion that the standard version (FLUKA 2011.2b) provides the best description of our experimental conditions.

A number of open questions remain which need to be clarified before the final analysis can be performed and the final results for the relative biological efficiency (RBE) of antiprotons stopping in biological media can be retrieved.

All these questions can be answered in-silico and no further data runs of AD-4 at the Antiproton Decelerator are foreseen. The AD-4 collaboration considers the experimental portion of AD-4 as closed and is diligently working on the final analysis and the preparation of a summary paper, describing the findings, well within the year 2014.

V. Acknowledgements:

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References:

1. L. Gray and T. E. Kalogeropoulos; IEEE Trans. Nucl. Sci. NS-29, p 1051 (1982)
2. L. Gray and T. E. Kalogeropoulos; Rad. Res. 97, pp. 246-252 (1984)
3. T. E. Kalogeropoulos and R. Muratore; Nucl. Inst. Meth. B 40/41, pp 1322-1325 (1989)
4. A. H. Sullivan; Phys. Med. Biol. 30, pp 1297 – 1303 (1985)
5. http://rsb.info.nih.gov/ij/
6. N. Bassler et al.; Phys. Med. Biol. 53, pp. 793 – 805 (2008)
7. G. Battistoni, S. Muraro, P. R. Sala, F. Cerutti, A. Ferrari, S. Roesler, A. Fassò, J. Ranft; AIP Conference Proceeding 896, 31-49, (2007)
8. A. Ferrari, P. R. Sala, A. Fassò, and J. Ranft, CERN-2005-10 (2005), INFN/TC_05/11, SLAC-R-773 (2005)
9. W. Hansen, *Experimental investigation of the suitability of the track structure theory in describing the relative effectiveness of high-LET irradiation of physical radiation detectors*, PhD Thesis; Risø National Laboratory, DK-4000 Roskilde, Risø-R-507 (1984)
10. N. Bassler et al.; Nucl. Inst. Meth. In Phys Res. B 266, pp. 929-936 (2008)
11. J. N. Kavanagh et al.; Eur. Phys. J. D60, pp. 209-214 (2010)
12. J. N. Kavanagh et al.; Scientific Reports (2013) DOI: 10.1038/srep01770