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Addendum 2

# AD-4 - Biological Effectiveness of Antiprotons

## Proposal for an Extension of Experiment AD-4.

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## I. Introduction

During the run period in 2004 we have shown a significant enhancement in the biological effective dose ratio (BEDR) between peak and plateau regions of a 50 MeV antiproton beam stopped in a biological target compared to experiments with protons using nearly identical beam parameters (CERN-SPSC-2004-031). The measurements have strongly supported our earlier, preliminary findings of a four-fold enhancement of the BEDR of antiprotons compared to protons, under the specific experimental conditions used for our experiments at the AD. In addition to these biological measurements we have initiated R&D on dosimetry and real time imaging.

Based on these results we propose to continue our cell-biological measurements with antiprotons with the goal to generate a complete data set suitable for benchmarking Monte Carlo calculations initiated by our group. These data will allow us to directly link our results to the work done in the area of heavy ion therapy, enabling us to perform a complete assessment of the feasibility of the potential use of antiprotons in medical applications with a minimum of antiproton beam time.

One of the most important issues in radiation therapy are side effects due to dose deposited in healthy tissue and critical organs nearby and late effects (secondary tumours) due to the total dose deposited in a patient. In antiproton annihilation a multitude of medium and high-energy secondary particles are produced and the question on side effects and secondary cancer induction is to be taken very seriously. Initial studies have shown no dramatic spread of biological effect beyond the primary beam, but more measurements, using a variety of biological endpoints and protocols are necessary to make a conclusive statement in this area.

In addition to these biological measurements we propose to continue R&D on real time imaging and continue our experiments and model calculations on physical dose deposition.

**II. Monte Carlo calculations.**

We are currently developing calculational tools based on three platforms: GEANT4, MCNPX, and SHIELD-HIT. GEANT4 has been developed as a tool specifically optimized for detector development in high energy physics experiments. Closer examination showed that the original version GEANT does not fully describe the details of the annihilation process and the range of secondary particles and their energy spectra. We are currently implementing specific modules to include recoil ions above helium and to describe their energy deposition in a biological target.

MCNPX is a general purpose Monte Carlo radiation transport code that tracks nearly all particles at nearly all energies. Version 2.5.0 is the latest generation in a series that began nearly sixty years ago. MCNPX is a formal extension of MCNP to all particles and all energies with improved simulation models; extensions of the neutron, proton, and photonuclear libraries to 150 MeV; and formulation of variance reduction techniques. The code is widely used throughout the world and has found particular use in medical physics for proton and neutron therapy planning.

Of particular interest for calculating the energy deposition of antiproton annihilation products is the handling of heavy ions from nuclear fragmentation. MCNPX correctly models the track lengths and energy deposition of light ions up through alpha particles (p, d, t, 3He, 4He). The code calculates the stopping and straggling of antiprotons and protons correctly. For heavier fragments the code calculates the total energy correctly but assumes it is deposited locally. In other words it does not explicitly follow the details of the "delta-rays" or high energy knock-on electrons produced by heavy ions. The code is able to tally the energy distribution of all annihilation products and such output is suitable for comparison to the other Monte Carlo codes.

SHIELD-HIT is a version of the SHIELD code especially adapted to the Heavy Ion Therapy program (Phys. Med. Biol. 49 (2004) 1933) and includes straggling and multiple Coulomb scattering, has built in stopping powers for H and He according to ICRU49 (1993), allows for more detailed energy grids and more exact interpolation of ranges and cross sections, and gives accurate track length estimation and fluences for all particles and fragments inside all target zones. SHIELD-HIT also allows to «switch off» various physical processes in order to obtain a better understanding of the dynamics of the reaction.

The energy deposition of light ions in biological targets and the production of fragments, both from the projectiles and from the target ions, have been benchmarked extensively using SHIELD-HIT. We have recently initiated discussions to join forces with colleagues from DKFZ in Heidelberg, Germany, GSI, Darmstadt, and the Institute for Nuclear Research of the Russian Academy of Sciences, Moscow to use SHIELD-HIT as a generator of a complete table of secondary particles and their energy spectra resulting from an antiproton beam stopping in a biological medium. These results can then be used as an input to the Local Effect Model (LEM) developed at GSI both to perform dose planning exercises and to calculate the response of thermo luminescent

detectors (TLD's) and Alanin tablets used in our earlier antiproton experiments. We expect these calculations to yield a reliable way of determining the physical dose deposited in cell targets, allowing the extraction of relative biological effectiveness (RBE) values. The knowledge of both physical dose and RBE can then be used in standard dose planning software for comparative studies of treatment plans for a specific tumor location and size using different radiation modalities (Photons, IMRT, protons, heavy ions, and antiprotons).

**III. Biological cell experiments at the AD**

**(a) Biological effect measurements in the primary beam channel.**

In order to benchmark these calculations we will need a complete set of experimental input data. We therefore propose to perform irradiations of standard cell lines (e.g. V79 Chinese Hamster) using an antiproton beam with a kinetic energy of 100 – 200 MeV, providing penetration depths between 75 and 250 mm. As most work in proton and carbon ion therapy has been performed in this (clinically relevant) energy range these measurements will be directly comparable to existing data using protons and light ions. In addition, the range straggling at such penetration depth will spread out the Bragg peak to a width which is compatible with the gel-slicing protocol successfully used in our past experiments and which is known to yield highly reliable RBE measurements in proton an carbon ion beams, alleviating the need to use a ridge filter to artificially generate a spread out Bragg peak (SOBP).

In preliminary discussions with the AD team it became apparent that a modest change in the AD cycle would allow for these higher energies, essentially up to the maximum energy of the DEM line design. The proposed solution consists of decelerating and cooling the antiprotons circulating in the AD according to the standard protocol to the plateau at 300 MeV/c. After electron cooling at this lowest momentum the beam could then be accelerated swiftly to the desired extraction energy and extracted to the target without further cooling.

An important issue in particle beam therapy with high LET is the possibility to successfully control radio-resistant, i.e. hypoxic tumours. Therefore an important experiment to be performed with antiprotons will be to irradiate a sample of a hypoxic cell culture and compare the results to our standard experiment, conducted during the same beam period under otherwise identical conditions.

**(b) Peripheral damage studies.**

A critical issue in particle radiation therapy, especially when considering antiprotons as projectiles, is damage done to cells outside the direct target volume. Such damage is unavoidable in the entrance channel, but can be mitigated by a high BEDR between peak and plateau regions, as hinted by our current results with antiprotons. Outside the direct beam path and beyond the distal edge of the Bragg peak such damage can occur by neutrons generated in nuclear interactions between projectile and target

atoms in the case of protons, by neutrons and fragments produced from projectile ions in the case of light ion therapy, and by the medium range annihilation products generated in antiproton reactions with target atoms. While these effects have been studied in detail for protons and light ions, both experimentally and by model calculations, the situation in antiproton annihilation is much more complex. A detailed experimental knowledge of the dynamics of the annihilation event is currently not available. We therefore decided in our previous run period to conduct an initial experiment looking for an integral biological effect outside the direct beam. (To avoid background from the beam halo we concentrated predominantly on the region distal to the Bragg peak). For these measurements we used the best available beam focus at our beam line to deposit a high dose in a small target volume. According to rough estimates of the dose, based on MCNPX calculations for the fluence of particles delivered and the beam profile measured at the entrance to the biological target we were able to reach a peak dose of close to 200 Gy in the Bragg peak. We then used the biological response of cells to study the effect outside of this target volume. Using both clonogenic survival and a biological assay able to detect damage to the DNA inside a cell nucleus (COMET assay) we did not detect a significant effect more than 3 – 5 mm away from the distal edge of the Bragg peak.

These initial observations are encouraging but not sufficiently quantifiable to make a conclusive claim. Radiobiologists strongly suggest to perform a measurement using a more realistically spread out Bragg peak covering a typical clinical volume of a few cubic centimeters. While the central dose would be limited, the physical dose deposition in the periphery will more closely mirror an actual treatment situation. In addition, within the ENLIGHT network protocols are being developed to address the questions of late effects. These protocols are based on the study of cell apoptosis and mutation induction, genomic instability, transformation, and enhanced terminal differentiation. We are currently attempting to obtain a draft version of these protocols and will design our antiproton experiments to match these accepted protocols. This will allow a direct comparison to data from light ions and protons.

We propose to perform the necessary measurements using our standard cell line (V79 Chinese Hamster) and the methodology developed during the initial run period. As this method has proven to produce quite accurate clonogenic data, continuing with the same method will alleviate the need for additional R&D with antiprotons. Test experiments to develop expertise in new protocols for the cell analysis can be performed off line using x-rays and protons. The only change in the experimental set up we propose is to use a higher energy beam (therefore obtaining a deeper penetration into the target) and to perform the majority of these studies using a spread-out Bragg peak to cover a volume of approximately 3 $cm^3$.

Assuming an initial energy of 100 MeV (450 MeV/c) the penetration depth in water would be approximately 75 mm with a width of the pristine Bragg peak of about 1.8 mm. Additional layers of degrading material can be added to produce the required Spread Out Bragg Peak (SOBP). Figure 1 shows an example for 100 MeV protons entering a water target. 7 different layers of plexiglass of 1 mm thickness were added sequentially and the resulting stacks were used for approximately 21, 13, 11, 8, 7, 6 and

4% of the overall irradiation time in addition to the 30% with no additional degrader. This results in a SOPB with less than 5% dose variation over a depth of 12 mm. It can be seen that the peak dose is reduced to about 45% in the SOBP compared to a pristine peak and accordingly a longer irradiation time is needed to reach the same dose level.

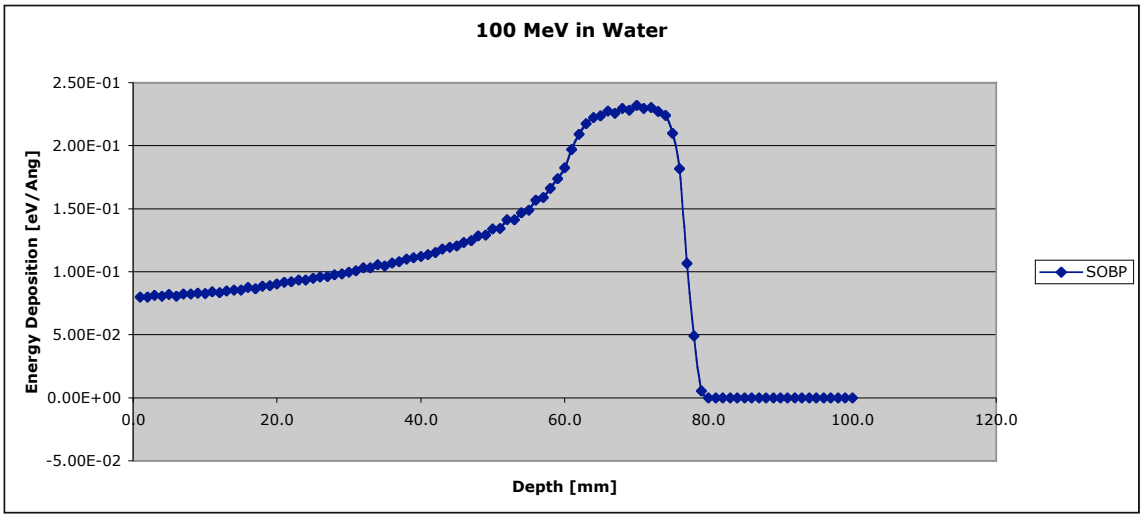


Fig. 1: SOBP generated by adding additional degrader material at 7 different thicknesses in the beam path. The variation across a depth of 12 mm is less than 5%. The calculation was performed using SRIM 2003 for protons at 100 MeV entering a water phantom.

Filling a spherical volume of a few cubic centimeters with a homogeneous dose distribution in both the axial and the radial directions will not be realistically achievable using a Gaussian beam. (To achieve a lateral homogeneity of 5% requires that 95% of the beam is outside the target diameter). Instead, we propose to use lateral spot scanning, where we aim for a small focus (r = 2 – 5 mm) and lateral displacement by magnetic deflection near the exit of the beam line to map out a larger circle. Based on MCNPX calculations scaled for the deeper penetration and the spreading of the Bragg peak we estimate that we should be able to reach a total of 45 Gy in the target region within a 24 hour irradiation period assuming standard AD operating parameters. This would allow us to complete a sequence of 1, 2, 5, 7, 10, and 20 Gy irradiations in a single 24 hour beam time and to study a situation more closely resembling a true treatment situation.

The beam would be sent into our standard water phantom which would be loaded with both a cell sample covering the entrance channel, the entire target volume (SOBP), and the distal region. In addition, cell samples would be placed outside the direct beam path in lateral direction. The available number of cell samples will be large enough to perform a variety of biological assays, studying different biological endpoints, for each experimental sequence. We propose to perform at least two independent sets of irradiations.

## IV. Dosimetry of antiproton beams.

Absolute dosimetry continues to be a main challenge to our experiments as well as to the development of antiprotons for potential therapeutic applications. This is caused

both by the pulsed structure of the beam currently available from the AD and by the complexity of the annihilation event producing a mix of particles. We have used fluence measurements, in-situ beam spot monitoring, and Monte Carlo calculations to predict a physical dose deposited in the target. For the plateau region these results can then be used together with the assumption that the biological effectiveness of high-energy antiprotons and protons is identical to confirm the calculations. For the peak dose values we need to rely on Monte Carlo calculations, which have been benchmarked against the few experiments on physical dose deposition available (i.e. A.H. Sullivan, 1985). Further studies, using GAF-chromic film, TLD's, neutron dosimeters, and other dosimetry instrumentation are necessary to provide more benchmark data to test current and future model calculations. Specifically, we plan to use SHIELD-HIT to generate a complete particle spectrum, each with its associated energy spectrum for the secondary particles, which can then be used in the Local Effect Model (LEM) developed at GSI to predict the response of TLD's and possibly Alanin tablets. We will use our initial data set at 50 MeV kinetic energy obtained in 2003 and 2004 as test cases for these calculations, but propose to augment these data by a detailed set of experiments at higher energy (thereby obtaining deeper penetration depth and higher spatial resolution).

**V. Real time imaging.**

Antiproton annihilation offers the possibility to detect the exact location of energy deposition in a target on a particle-by-particle basis, in real time. This possibility is considered by many members of the oncology community as the second most important advantage of antiprotons over protons, aside from the enhanced biological ratio, as often in treating small tumors near sensitive regions of the human body a higher precision in matching the energy deposition to the known tumor shape is needed. We have performed first proof-of-principle experiments using two different types of detectors, and propose to continue the studies to establish proper measurements for the resolution achievable and the minimum number of antiprotons needed.

Using a pristine Bragg peak with a penetration depth of 75 mm or above we will attempt to detect the axial dose distribution due to annihilation of antiprotons in flight. Based on known cross sections for in-flight annihilation we can calculate an intensity profile for these events along the beam axis and thereby establish both the minimum number of events necessary for unambiguous detection and the relationship between event number and spatial resolution with a single measurement. To study the spatial resolution with high precision we propose to use targets with specific 3-D shapes to stop antiprotons delivered from the DEM line. By introducing air gaps in the target we can generate sharp transitions between regions of annihilation and regions were the event number is minimal. We propose to perform these runs using the two types of detectors used in the test runs in October of 2004 to detect either the high energy gamma's or the charged pions. In the latter case two or three planes of detectors will be used to reconstruct the tracks, in the case of the gamma's we will use a shadow mask method to generate 2-D projections of the target from at least two different directions.

The goal of the experiment is to establish a resolution limit of the detection method, including straggling in surrounding materials and secondary production in the

masks (for the gamma case) as well as establish the minimum irradiation dose needed for reliable detection of the irradiated volume. It is assumed that a very low, pre-therapeutic dose will be sufficient to establish conformality with the prescribed "tumor" volume.

## VI. The AD-4 Collaboration

The original set of collaborating institutions in AD-4 continues to be the central core of our team. During 2004 we added a few additional members to address specific questions. A list of current members of the AD-4 collaboration and their affiliations is given in Appendix II.

In addition to the core collaboration we have initiated joint efforts in the area of model calculations with DKFZ (O. Jaeckel, O. Filipengo) and INR (N. Sobolevsky). While these colleagues are not an official part of AD-4 at this time, their efforts will substantially enhance our portfolio. We have had several discussions with Prof. Kraft and his group at GSI and plan to submit a joint proposal for beam time at GSI to perform carbon comparison experiments. In the area of real time imaging we have secured support and equipment through BioScan, SA in Geneva for the high energy gamma detector and with members from the ALICE detector development team for the pion vertex detector.

To strengthen our position at the Geneva Hospital, where we have performed most of the recent cell preparation and analysis work, we have invited Dr. Oliver Hartley from CMU to join our collaboration and the details are currently being discussed with his administration.

Dr. Vera Garaj-Vrhovac from the Institute for Medical Research and Occupational Health in Zagreb was instrumental in the early tests for DNA damage and we expect that she and her group will join our collaboration if a continuation of the work is approved. To this end our collaborators from Serbia-Montenegro and Aarhus have invited her to join them in applying for EU funding of this work. The first step was successful and the pre-proposal has been selected to submit a full proposal by the end of September 2005.

## VII. Summary

Current results from biological measurements at the AD during the run periods in 2003 and 2004 serve as motivation to continue our studies to obtain a more precise data set to produce a complete assessment of the feasibility of the biological use of antiprotons. The main issues to be covered are RBE measurements, peripheral effects (and possibly late effects) outside of a clinically relevant volume, absolute dosimetry, and real time imaging. We will develop calculational tools based on SHIELD-HIT, MCNPX, and GEANT4 to address these issues, but experimental data in all areas are needed for benchmarking our codes and to address direct, face-to-face comparisons with alternate treatment modalities already used in radiation therapy.

**APPENDIX I:**

**Specific request for technical modifications and beam time:**

To obtain a deeper penetration into the target and to allow a better direct comparison to data obtained with carbon ions and protons a kinetic energy of 100 – 150 MeV is needed. This can be achieved in the current DEM line by decelerating the antiprotons in the AD to 300 MeV/c, perform electron cooling at this standard plateau, and then reaccelerate the beam to the desired extraction energy prior to extraction without any further cooling. Informal discussions within our collaboration and with the AD team indicate that this is technically feasible and will require none or minimal hardware upgrades. We will provide the necessary manpower for design calculations from our collaboration and will support the AD team in tests and commissioning of this method.

We estimate that the complete set of experiments described in this proposal will require approximately 200 hours of beam time.

Biological experiments are complicated by the fact that cell preparation and analysis present major organizational challenges and strict time windows have to be adhered to. In addition, as biological response can vary from experiment to experiment it is required that one complete set of irradiations is completed in a single run, so calibration by Co-60 gamma rays can be performed. It has been suggested that the requested mode running at higher energy would be sufficiently different from the standard AD operation and that the beam time for AD-4 should be lumped into fewer time blocks than before. We therefore request two blocks of beam time, separated by at least 6-8 weeks for analysis and preparation of the next set of experiments.

Each of the two time slots would be used to perform a full set of biological irradiations and as many R&D on dosimetry and imaging as time allows. Assuming 24-hour operation of the AD we propose to split the 200 hours into two sets of 4 days each.

Some specific tests can be performed under nominal operating conditions from the AD (extraction at 300 MeV/c). Therefore, as always, we will prepare a set of short experimental studies to take advantage of any beam time offered by other experiments due to technical problems.

**APPENDIX II:**

**The AD-4 Collaboration**

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