the growth of newly formed hybridomas.[15,39-41] The work performed on this aspect in the 1980s has culminated with the discovery that a characterized plasmacytoma/hybridoma growth factor[42,43] was identical to interleukin-6 (IL-6), which is a pleiotropic cytokine active on the differentiation of B lymphocytes.[44] The importance of adding exogenous IL-6 (or medium conditioned by IL-6–producing cells such as the P388D1 monocytic cells) to hybridoma cultures was further shown by the observation that IL-6 preferentially promoted the survival of the hybridomas secreting MoAbs.[45] The dependence to IL-6 is rapidly lost during prolonged cultures.

Another important aspect of the culture of newly formed hybridomas is the quality of the fetal calf serum used. The quality parameters are not yet defined, but it is clear that certain serum lots are much better than others for promoting the growth of newly formed B cell hybridomas. The relative performance of the serum lots should be tested in a fusion experiment rather than by their effect on the growth of myeloma or established hybridoma cells.[45]

Source of Antigen-Activated B Lymphocytes

It is quite clear that the activation of antigen-specific B lymphocytes is a parameter that is much more important than the immortalization step. As a matter of fact, myeloma cells fuse efficiently only with activated B lymphocytes that are at a differentiation stage somewhere between resting B lymphocytes and fully differentiated plasma cells.[46] Preparation of B lymphocytes at the appropriate differentiation stage is relatively easy in the murine system but represents a major problem in human hybridoma technology.

Murine Lymphocytes

The routine protocol for preparing murine B lymphocytes suitable for efficient fusion is to immunize a group of mice with the antigen of interest, determine the serum antibody level in the individual animals, select the animal showing the highest antigen-specific response, and recover the activated spleen cells 3 or 4 days after a last injection ("boost") of antigen.[15] This basic protocol has been used since the original description of the hybridoma technology and has been successful thousands of times. However, it is not unerring, and all investigators in this field have experienced failures most probably caused by the absence of antigen-activated lymphoblasts in the spleen cells recovered on the fusion day. Many of these failures may be caused by the induction in the animal immune system of a suppression/tolerance state preventing the final activation step. The possible reasons for this phenomenon are diverse and may include such parameters as seasonal variations (springtime is not very good) and the pampering of animals. One measurable parameter that may cause this phenomenon is the immunization protocol; we have observed that hyperimmunization of mice was not advisable for the preparation of a high number of antihuman red blood cells MoAbs.[47] Another parameter that is relevant to transfusion medicine concerns the optimal time period between the final antigen boost and the fusion experiment. We have previously shown that the optimal period may be shorter (2 instead of 3 to 4 days) for some antigens such as the blood group A antigen.[48]

Although several factors influencing the yield of antigen-activated B cells are still not well defined, it is clear that most problems can probably be avoided by using groups of mice and various immunization schedules.

Human Lymphocytes

The lack of a suitable and reproducible source of antigen-activated human B lymphocytes has been and still is the major difficulty in the preparation of human MoAbs by the hybridoma technology. The reasons for this are multiple. Obviously, humans cannot be immunized with antigens of interest. Furthermore, the peripheral blood was until recently (discussed subsequently) the only routinely available source of human B cells (at least to blood banks).

In vitro immunization and activation. In the 1980s, much effect was made in the development of conditions favoring the in vitro immunization and activation of human peripheral blood lymphocytes (PBL). Such systems have been described[49,50] and human MoAbs have been prepared from the B cells activated in vitro.[51-54] However, it is generally recognized that the efficiency of such systems is low compared with murine in vivo systems. In transfusion medicine-related work, several laboratories (mostly in Europe) have been successful in the preparation of human anti-Rh antibodies by using in vitro–activated (pokeweed

end of December 2000 (each patient had made measurement once or two or three times). Semiconductor detectors (with DPD 510 by Scanditronix) were used during *in-vivo* dosimetry. Doses were calculated and measured in (1) the centre of the irradiation field; (2) supraclavicular region; (3) mediastinum; (4) lower edge of the field and (5) neck. Patients were irradiated at various accelerators, most of them at Neptun with photons 9 MeV.

Results: All patients were divided into three groups. The criterion of inclusion was the per cent difference between calculated and measured doses average for all dosimetrical points. The ranges for the groups were: 0-5%, 5-10% and over 10 %. The mean per cent differences in the first group of 43 patients was 3.1%, in second of 27 patients – 6.3%, and in third of 6 patients - 17.6% respectively. There was no clear reason, beside an accidental error why for the certain patient difference was much larger than for the another. Mean difference for all groups was equal to 5.3%.

In the table mean per cent differences between doses calculated and measured and their standard deviations (SD) in the whole group of patients are shown for central axis, mediastinum and supraclavicular region.

central axis		mediastinum		supraclavicular region	
Mean diff.	SD	Mean diff.	SD	Mean diff.	SD
1.5%	4.1%	-0.3%	4.6%	2.0%	5.7%

Conclusion: Mean difference in the whole group of patients shows good agreement between pre-calculated and measured doses, especially for three clinically important regions (table). It is accompanied by low standard deviation which is an indicator of small deviations between doses inside the whole group.

6.
THE TECHNIQUE OF TOTAL BODY IRRADIATION APPLIED IN THE ST. LESZCZYŃSKI MEMORIAL HOSPITAL IN KATOWICE

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At the St. Leszczyński Memorial Hospital in Katowice a modification of *TBI* technique was prepared. For this a special two variant of body frame - one for treatment planning and an another one for treatment delivery - was made. The total dose of 12 - 15 Gy (in lung not more than 9 Gy) was delivered in six fraction of 15 MV photons, produced in Primus linear accelerator, for 3 consecutive days. Patient was treated by a combination of fields: lateral - set at *SSD* of 330 cm and *AP/PA* - set at 135 cm. The dose-rate measured at 10 cm in a water phantom for lateral fields was 4,3 cGy/min., and for *AP/PA* fields 23,6 cGy/min. Lung shields were made from wood alloy and their shape was carried out from computerized tomograph scans (*CT*). For each patient a set of computerized tomograph scans was prepared. Patient during the *CT* was laying in supine position in the body frame made of 1 cm thick plexi plates. On the walls of that body frame a special marks of tin material were inserted. These marks allow to reproduce both - the same patient position during the irradiation and also in the treatment planning system *HELAX.* Position of shields before *AP/PA* fraction was determined by means of *HELAX,* and then shields were fastened to plexi trays inserted in the head of Primus. Lung was also shielded during one lateral fraction and the shape of the shield was carried out on a simulator. The volume between the patient and walls of the body frame was fulfilled by bolus (bags with rice) to get a homogenous dose distribution. The electron boost to the thorax wall (shielded for 15 MV photons) was delivered with a 6 or 9 MeV electron beam.

The percentage deviation of dose, for all 9 irradiated patients, calculated at ten anatomical points representative of the body anatomy, was in the limit -0,4% to +13% (excluded in lung) from the dose delivered to *PC* (reference point: 1/2 *AP* and 1/2 lateral dimension at 1/2 of patient length in irradiation position). The *in vivo* measurements carried out by means of MOSFET detectors confirmed that accuracy.

7.
IMRT – NEW STANDARDS IN TREATMENT PLANNING

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Technological advances in medical imaging have prompted accelerator manufactures to produce more and more advanced treatment delivery systems capable to precise shape the dole distribution.