Standard Operating Procedures.

Chemosentitivity testing *in vivo*

All experimental procedures are carried out in full compliance with the UKCCCR Guidelines on the Welfare of animals in experimental neoplasia. (Revised June 1997)

Determination of antitumour activity in mice.

Experimental procedures are identical whether syngeneic tumours or human tumour xenografts are being used. The timing of individual procedure will of course vary with the tumour growth rate.

Transplantation of Subcutaneous tumours.

a. Solid Tumours.

Healthy donor animal with "actively growing"* tumours are selected, humanely killed, usually cervical dislocation and under full aseptic conditions tumours removed and placed in petri dishes with tissue culture medium (e.g. RPMI 1640) without antibioitics.

Approximately 2x2 mm cubes of viable tumour are cut up using a scalpel and forceps.

A single tumour fragment is transplanted subcutaneously in the flank using a trocar. In general only one tumour per mouse is used. If bilateral implantation is used, then the total tumour load must not exceed that in the Guidelines.

b. Cell suspensions.

Cells from logarithmically growing cultures are harvested and resuspended in fresh medium, (without antibiotics) at a concentration so that the intended inoculum may be administered in a volume of 0.2ml. e.g. 10^7 cells/ml = 2×10^6 inoculated.

Cells are inoculated subcutaneously in the flank using a 26g 25mm needle to minimise leakage.

Animals are checked regularly for signs of tumour growth, (experience will determine the time intervals). Those receiving cell suspension are also checked for signs of ascites. Any animals showing such signs are humanely sacrificed.

Once tumours reach a size where they can be reliably measured with callipers, (e.g. 3x4mm) animals bearing suitable tumours, i.e. with an acceptable size range (define) they are allotted into appropriate groups by restricted randomisation. The number of animals per group in kept to the minimum required for statistical analysis. (This may vary with the tumour characteristics but usually between 5 and 8 per group is sufficient).

Mice are then marked e.g. ear clipping, tumours are then measured with callipers and the volume calculated (a mm^2 x b mm/2) = vol. in mm^3. This is the pre-treatment volume and is taken to determine that all tumours are growing prior to treatment. Experience will determine the timing of the pre-treatment measurement, usually 2 -4 days will be required. Tumours that do not grow in this period are rejected.

Treatment begins on Day 0. Solvent control as well as non-treatment controls are included if toxic solvents are used, e.g. DMSO. All tumours are measured and all animal weights recorded. Solvents and drugs are given by the chosen route, animal weights and tumour volumes are then measured at regular intervals. Mean tumour volumes are calculated and related to the mean starting volume, to give a relative tumour volume, RTV. Tumour growth is followed for a sufficient period to time to produce meaningful growth curves, tumour volumes are not allowed to exceed those given in the guidelines, e.g. in mice where the mean diameter must not exceed 17mm. Once tumour reach this size, animals are humanely sacrificed.

Mice showing signs of ill health or excessive weight loss, 10%, are also humanely killed.

At the completion of the experiment, antitumour effects are calculated in terms of tumour growth delay (days) at a standardised RTV.

Statistical significance of antitumour effects are determined by an appropriate test, e.g. Mann-Whitney.

* As determined by previous experience.