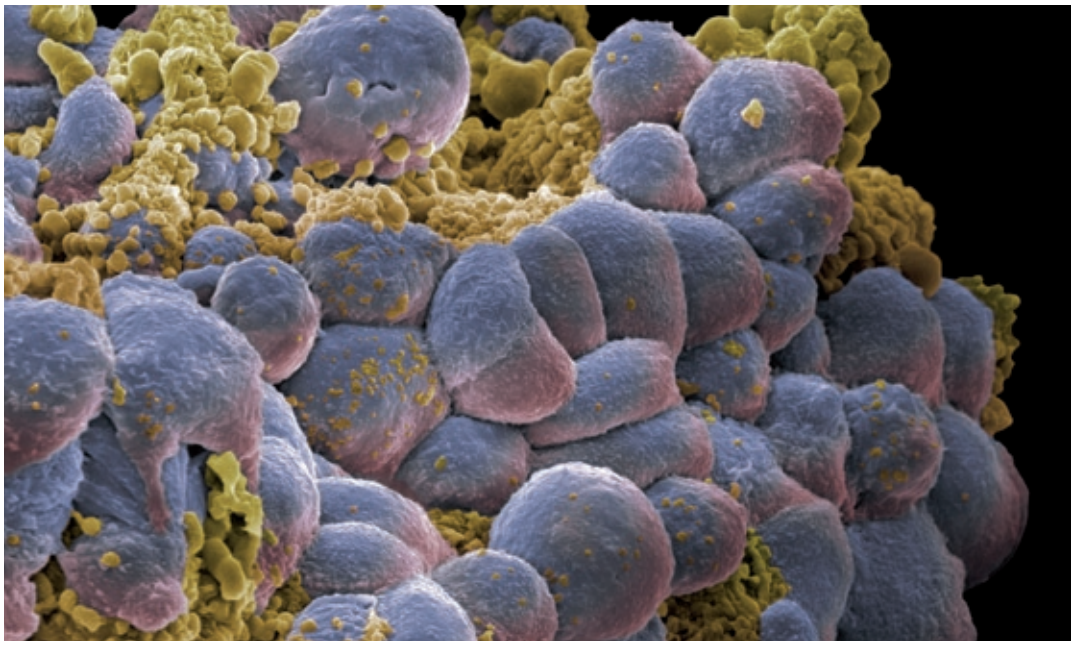
SAFE CELL-BASED THERAPY



TNO triskelion bv

Tumorigenicity testing helps you assess the safety of your cell substrate, bioactive protein or vaccine and ensure that subjects enrolled in clinical trials involving cell based products are not exposed to significant and unreasonable risks. TNO tailors tumorigenicity studies to meet your requirements, while operating within the framework of the relevant regulatory guidelines.

Tumorigenicity is defined as the process by which cells form tumours when inoculated into animals (usually an immunosuppressed xenogeneic host). The tumorigenic phenotype of the cells may change as a result of the genetic modifications with the specific human production genes, caused by altered cell culture conditions needed for master cell banks and working cell banks or as a result of up-scaling of the fermentation process to production level (End of Production Cells). An increased tumorigenic phenotype may occur, for example, as a result of clonal selection due to prolonged serum free culture. For production cells of human origin, the FDA and EMEA may require data on the tumorigenic potential at different stages of the preclinical and clinical development of the biopharmaceutical product or the advanced therapy (gene or cell therapy or tissue engineering product). The initial tumorigenic potential of novel cell substrates can be evaluated at a relatively early stage (e.g. before phase-I clinical trials), supplemented by additional, less extensive tumorigenicity testing at later stages in development (e.g after establishing master and working cell banks and up-scaling of the GMP production for phase III clinical trials). Since there is generally a lack of data for new production platforms, this iterative process helps establish the safety of the new technology. Each preclinical testing strategy should be discussed in advance with the regulatory authorities on a case by case basis.

TNO offers advice on when tumorigenicity studies are required and offers GLP compliant tumorigenicity testing of cell substrates in athymic nude mice according to FDA guidelines and European Pharmacopoeia. The TNO study outline for a tumorigenicity study is as follows:

Test species: athymic nude mice of one sex (usually females)
Treatment: negative control (MRC-5 cells), positive control (HeLa cells) and test cells
Dose route: subcutaneous inoculation with cell suspensions in serum-free medium
Group size: 10 - 30 animals per group
Housing: HEPA filtered individually ventilated cages (1 - 3 mice per cage)
Dose groups: low-, mid- and high-dose level for each treatment
Dose levels: 103, 105 and 107 viable cells per mouse
Dose volume: 0.2 mL per mouse
Study duration: 12 to 20 weeks
Monitoring: mortality, morbidity, clinical signs, onset of tumours, growth of tumours (in 2 dimensions)
Necropsy: several organs, injection site, draining lymph nodes, tumours (including metastases). Necropsy will be performed avoiding contamination of tissue samples with human DNA
Parameters: onset of tumours, size of tumours, TPD50 (Tumour Producing Dose at which 50% of the animals develops a tumour), microscopic evaluation of tissues and tumours, PCR analysis of tissue/ tumour samples for species confirmation (man or mouse?)

We have gained extensive experience using this model for several clients. Table 1 provides an overview of our track record and typical results in terms of tumour incidences. It shows that our negative controls are always negative and that the tumorigenic phenotype depends on the inoculated dose even for the positive control cells. This indicates that the multiple dose level design may be preferred, especially for weakly tumorigenic cells. The test cells in Table 1 are all derived from human tumour cells.

TNO handles studies up to 400 animals. The final study design is determined in close consultation with the sponsor. The study complies fully to GLP regulations. Due to the GMO nature of the cells, a license from the Dutch authorities is needed. TNO can apply for a separate license for each GMO, if necessary.

Cell culture, preparation of the doses for inoculation (including quality control) and timely delivery of the test suspensions to TNO is usually the responsibility of the sponsor. At your request, we can offer the cell culture services as well, but not under GLP. Previously, non-GLP cell culture has been accepted by the FDA.

The multi dose-level design of TNO's tumorigenicity studies provides information on the tumorigenic potential of the cells in relation to the known tumorigenicity of a relevant positive control cell line. In addition to characterizing the tumorigenic phenotype of live cells, TNO can test the oncogenic properties of cellular DNA extracts or cell lysates. For cell DNA and lysates, animals with a normal but immature immune system are normally used (newborn hamsters, nude mice or rats). The 20 week study duration is considered sufficient for determining the latency time for tumor development. The costs for tumorigenicity studies highly depend on the study design (no. of animals: 90 - 360, study duration: 12 - 20 weeks; species confirmation analysis etc.).

We invite you to contact TNO for further information and cost estimates.

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Table 1. Track record of tumorigenicity studies (tumour incidence).

	MRC-5 cells			Test cells			HeLa cells		
	10^3	10^5	10^7	10^3	10^5	10^7	10^4	10^5	10^6
Test 1 (20 weeks)	0/10	0/10	0/10	0/30	0/30	15/30	0/10	0/10	8/10
Test 2 (20 weeks)	NT	NT	0/10	0/30	0/30	8/30	NT	NT	9/10
Test 3 (20 weeks)	NT	NT	0/10	0/30	0/30	29/30	NT	NT	4/10*
Test 4A (12 weeks)	0/30	0/30	0/30	0/30	0/30	7/30	0/30	0/30	25/30
Test 4B (12 weeks)				0/30	1/30	28/30			
							10^3	10^5	10^7
Test 5A (20 weeks)	0/30	0/30	0/30	0/30	0/30	2/30	0/30	1/30	30/30
Test 5B (20 weeks)				0/30	0/30	8/30			
			10^7			10^7		10^6	10^7
Pilot (6 weeks)			0/10						10/10
Pilot (20 weeks)			0/12			12/12		12/12	

NT = Not Tested
** dose was 2x10^5 cells due to a dilution error*

TNO TRISKELION BV
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