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Comment on: 'Guidelines for the use of cell lines in biomedical research': human-to-human cancer transmission as a laboratory safety concern

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Sir,

The guidelines for using cell lines in biomedical research, published recently in *BJC* (Geraghty *et al*, 2014), include a pioneering safety warning about human-to-human cancer transmission through cancer cells, a route that we will call extracorporeal metastasis (XM). Because XM has been tacitly dismissed as implausible in everyday laboratory practice, if considered at all, we emphasise the warning by reviewing the underlying evidence and preventive measures.

The possibility of XM was suggested 50 years ago (Bloom *et al*, 1951) and confirmed by finding tumours that are transplantable in outbred mice (Eiselein and Biggs, 1970; Fidler *et al*, 1981). In wild animals, XM has been documented for dogs (Murgia *et al*, 2006; Rebbeck *et al*, 2009; Belov, 2011), in which cancer is sexually transmitted (Rebbeck *et al*, 2009), and Tasmanian devils, in which it is passed by biting (Murchison *et al*, 2012). Although the devils are highly inbred, dogs are not, implying that transmitted cancer cells evade the immune response. Indeed, they do so through the loss of major histocompatibility complex proteins, thus preventing humoral immune response, and by increasing TGF-β expression, which protects from natural killer cells (Siddle and Kaufman, 2013). Because evading the immune surveillance is also common to human cancer (Cavallo *et al*, 2011), it should not come as a surprise that XM happens in humans, albeit under particular circumstances.

The possibility of XM in humans was first tested half a century ago in experiments that now appear medieval and that involved inoculating human cancer tissues or cancer cell lines into healthy individuals or into cancer patients (Moore *et al*, 1957; Langer, 1964; Brunschwig *et al*, 1965). Most inoculates failed to survive but some persisted, metastasising into local lymph nodes or recurring after the primary tumours caused by the inoculation had been excised (Moore *et al*, 1957; Langer, 1964). In another experiment, a slice of melanoma transplanted from a patient into her 80-year-old mother killed the recipient 451 days later by disseminated metastases, although the initial implant was resected 21 days after the implantation and the patient was treated with chemotherapy (Scanlon *et al*, 1965).

The implications of these now-unthinkable experiments became clear once organ transplantation became common and XM (especially by melanoma) through transplanted organs became a serious problem, as even organs free of overt cancerous tumours can still transmit cancer, apparently by harbouring disseminated or circulating cancer cells from the donor (Strauss and Thomas, 2010; Desai and Neuberger, 2014). This problem has been minimised by screening donors, but not yet eliminated (Desai and Neuberger, 2014). The risk of XM does not seem to apply to blood transfusion from donors who previously had cancer, at least to immunocompetent recipients (Yang *et al*, 2010), perhaps because cancer cells do not survive or adhere to the plastic containers during processing and storage of blood (Matsui *et al*, 1989; Simanovsky *et al*, 2008; Brennen *et al*, 2013).

Unfortunately, XM is not limited to immunocompromised individuals and does not require organ transplant to occur. In one reported case, a sarcoma was transmitted from a patient to the surgeon who pricked his hand during surgery (Gartner *et al*, 1996). The transmission was noticed and documented only because the pathologist who examined the patient's tumour also happened to examine the surgeon's tumour and noticed that their histopathology was remarkably similar, which prompted the investigation (Gartner *et al*, 1996). A similar accident occurred in a laboratory at the National Institutes of Health (USA), when a healthy young woman accidentally pricked her hand with a needle 'that had been previously used to draw up a suspension of a human colonic adenocarcinoma cell line' (Gugel and Sanders, 1986). The wound was superficial, but 2 weeks later it produced a nodule formed by the adenocarcinoma cell line. Remarkably, the nodule showed no signs of inflammation (Gugel and Sanders, 1986), highlighting the ability of cancer cells to avoid immune surveillance. Such accidents—pricking yourself with a needle or scalpel previously exposed to cancer cells—are not common, but by no means extraordinarily rare in the operating room or the laboratory, implying that these two reported cases of transmission may be exceptional only in that XM was noticed, documented and communicated to warn the broader biomedical community.

Besides pricking accidents, it is reasonable to assume that other routes used by infectious agents can also enable XM. These include the cracks on the skin, entering through the eyes, which might be particularly vulnerable because of the limited activity of the immune system (McKenna and Chen, 2010), and inhalation of aerosols, which are commonly formed while handling cells and have been documented as a route of cell line cross-contamination (Torsvik *et al*, 2010). Although inhaling cells while handling them in tissue culture hoods is highly unlikely, as the hoods are designed to prevent this possibility, cancer cells are routinely collected and processed outside the hoods, at which point they are considered as merely a reagent rather than an organism that can invade a human.

In our experience, the possibility of XM is generally unknown to laboratory researchers, as it is not reviewed during their safety training, or is dismissed as implausible. Yet, without awareness, the risk of accidental XM in the laboratory may increase in the future as more cancer cell lines are established, and the lines that are already in use continue to evolve. Thousands of human cell lines have been established over the last 50 years (Barretina *et al*, 2012) by explanting cancer tissues, which implies selection for new properties. In addition to natural selection, the diversity of cancer cell lines has been further increased by routinely modifying them genetically. At the same time, the ability of cells to evade the immune response is usually tested only in the studies that are concerned directly with this question.

Out of an abundance of caution, we propose two actions to minimise the risk of XM in the laboratory. First, the notion that cancer cells themselves are possible pathogens should be included into routine laboratory safety training. Second, cancer cell lines, perhaps starting with those provided commercially and by cell banks, should be tested *in vitro* for their ability to evade immune responses in humans. The lines that show potential for immune evasion should be labelled accordingly and used with all due care.

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Comment on: 'Evaluation of chemoresponse assays as predictive markers'

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Sir,

We read with great interest the recent Short Communication by Korn and Freidlin (2015), which considers hypothetical examples challenging the 'match/mismatch' analysis presented in Tian *et al* (2014). In Tian *et al* (2014), we proposed and applied a novel match/mismatch analysis approach for evaluating the predictive value of a chemoresponse assay from an observational study, by investigating the assay's association with outcome. The match analysis was performed using the assay result for the administered therapy (assayed therapy = administered chemotherapy); the mismatch analysis was performed using the assay result for a randomly selected therapy from all assayed treatments for a given patient, not necessarily matching the administered therapy (assayed therapy ≠ administered chemotherapy). If the match association is stronger than mismatch association, then the association is potentially drug specific and the assay may have predictive value. Using three examples in which a hypothetical chemoresponse assay is assumed to have only prognostic value, Korn and Freidlin (2015) have indicated that this analytical method may incorrectly conclude that the assay has predictive properties.

We agree with Korn and Freidlin (2015) that the match/mismatch method employed in Tian *et al* (2014) should be applied in limited circumstances and likely cannot be generalised to all chemoresponse, or more generally to all predictive biomarker assessment studies. As Korn and Freidlin (2015) point out, in situations where either (1) the treatments being considered have meaningful differences in efficacy in the unselected population or (2) specific treatment selection for a given patient is based on factors that have prognostic importance, the match/mismatch approach is inappropriate. However, we believe that neither of these cases are present in the clinical situation of recurrent ovarian cancer considered in the study by Rutherford *et al* (2013).

Specifically, in their hypothetical examples 2 and 3, Korn and Freidlin (2015) assumed different efficacies across treatments. This is inconsistent with the clinical situation in recurrent ovarian cancer (to which the match/mismatch analysis was applied), where more than ten different drugs are recommended, but evidence from clinical trials fail to demonstrate that any one is superior to any other (National Comprehensive Cancer Network, 2014). In their hypothetical example 1, Korn and Freidlin (2015) assumed similar treatment effects for drugs A and B, but they also assumed that the patients treated by drug A were different from those treated by drug B in terms of patient prognostic profiles. In Korn and Freidlin's (2015) example, due to differences in subpopulations (pattern of assay results and sampling fraction can also be different), the match/mismatch analysis method is indeed inappropriate. However, in the study by Tian *et al* (2014), 15 drugs were evaluated and, as such, the heterogeneous pattern of assay results across treatments was far more complex than Korn and Freidlin's (2015) example that included two drugs. In addition, although it is possible that the treatment groups differ in prognostic profile, it is more likely, as demonstrated in clinical practice, that patients with similar prognoses have multiple therapeutic options, and there are no clear prognostic factors which dictate treatment decisions for individual patients. Taking all of these considerations together, after resampling, the likelihood that patients included in the mismatch analysis have similar prognostic profiles (on average), compared with those included in the match analysis, is quite high. Table 1 shows the comparison of patient prognostic profiles between match and mismatch analyses in the study by Tian *et al* (2014), demonstrating strong similarity between the two analysis groups. For the mismatch analysis used in Tian *et al* (2014), patients with heterogeneous patterns of *in vitro* response were assigned either 'sensitivity (S)' or 'resistance (R)' assay results by resampling. For match analysis, 28.6% were treated with an S drug and 71.4% were treated with an R drug, with mean multiple drug

Table 1. Comparison of prognostic profiles between *match* and *mismatch* analyses (sensitivity *vs* resistance)

	Match analysis		Mismatch analysis[a]	
	Sensitivity (28.6%)	Resistance (71.4%)	Sensitivity (25.2%)	Resistance (74.8%)
MDRI[b] (mean)	0.68	0.10	0.71	0.11
Age (mean, years)	57.3	63.3	58.9	62.5
ECOG PS (%)				
0	68.0	70.6	69.8	71.0
1 or 2	32.0	29.4	30.2	29.0
Cell type (%)				
Serous	65.3	69.0	65.9	69.8
Others	34.7	31.0	34.1	30.2
Tumour grade (%)				
1 or 2	15.9	23.3	17.9	23.1
3	84.1	76.7	82.1	76.9
TFI[c] (%)				
<6 months	38.7	47.1	38.9	47.0
⩾6 months	61.3	52.9	61.1	53.0

Abbreviations: MDRI = multiple drug response index; TFI = treatment-free interval.
[a]Mismatch analysis: results representing the averages of 3000 simulations.
[b]MDRI representing the percentage of all assayed therapies to which a patient scored as sensitive.
[c]TFI defined as the time interval from the end of treatment until disease progression in the first-line treatment setting.