Soccer is one of the most popular sports in the world. To control the game, referees issue yellow cards to players—often very exciting and passionate players—who have acted inappropriately. The yellow card indicates caution; any player receiving two yellow cards in a game is ejected. The equivalent of yellow cards (warnings) and red cards (banning) are also observed in medical research, practice and therapies; this is especially true for new approaches to mitigate any detrimental effects.

In an article just published in the *Journal of Molecular Cell Biology*, a new journal established by the Chinese Academy of Sciences and Oxford University Press, Li et al. (2009) raised concern about the safety of nanoparticles. The investigators provided experimental evidence that polyamidoamine (PAMAM), a group of materials that appear to be very promising as nanocarriers for drug delivery, can induce autophagic cell death in culture and *in vivo* in an acute lung injury (ALI) model in mice; and most importantly contributes to mortality. The administration of an autophagic inhibitor decreased the PAMAM-induced lung injury and mortality.

Li and colleagues have, in essence, given a yellow flag to the use of nanoparticles in medicine; the important message is that we must be cautious with respect to safety, although we explore the exciting potential of nanotechnology in medicine.

This is not the first study to address the safety of nanomaterials in the lung. In a recently published review article, Card and colleagues summarized potential imaging, diagnostic and therapeutic applications of nanoparticles in the lung (Card et al., 2008). The authors pointed out that several groups of nanomaterials (i.e. carbon nanotubes, carbon black, fullerenes, silica, metals or metal oxides) can induce inflammation and/or fibrosis in the lung. These nanoparticles were either directly introduced into the lung through inhalation, intranasal or oropharyngeal aspiration, or indirectly translocated after systemic administration (oral, dermal, intravenous, etc.).

The mechanisms of toxicity vary with the different types of material. Li and colleagues demonstrated that PAMAM induced cell death in A549 cells (a human lung carcinoma cell line); there were features of autophagic cell death, but this was not associated with caspase-3 activation (Li et al., 2009). Increasing evidence suggests that one mechanism by which cancer cells are resistant to chemotherapeutic agents is the lack of caspase expression and/or activation. Caspase-independent cell death is a relatively new area of research (Guicciardi et al., 2004). For example, lipopolysaccharide can induce A549 cell death by activation of the lysosomal protease cathepsin B, independent of caspase activation (Tang et al., 2006). Autophagy is another type of caspase-independent cell death which acts through regulation of Akt, a downstream target of phosphoinositide 3-kinase (PI 3-kinase). Akt is an important molecule that controls cell survival and promotes cell cycle progression and proliferation. In the current study, Li and colleagues demonstrated that PAMAM reduced phosphorylation (a sign of activation) of Akt and its downstream molecules, mTOR and S6. They used siRNA to further demonstrate the involvement of TSC2 as a mediator in the Akt-TSC2-mTOR pathway of autophagy induced by PAMAM (Li et al., 2009). These molecular studies support the concept that the type of cell death induced by PAMAM is autophagy and, importantly, provide plausible targets for future interventions. A word of caution, however, is that the study by Li and colleagues did not provide *in vivo* evidence of autophagy, and the dose of PAMAM used was lethal, which makes interpretation of the results less clinically relevant. These issues should be addressed in future studies.

ALI and its most severe form, the acute respiratory distress syndrome, are life-threatening conditions affecting approximately 1 million people world-wide annually. The importance of ALI has been highlighted by the emergence of SARS (severe acute respiratory syndrome),
avian flu and the recent H1N1 influenza pandemic. Cell death, in particular necrosis and apoptosis, has been recognized as an important mechanism of ALI. Recent investigations have demonstrated that there are multiple ways for cells to die during lung injury (Tang et al., 2008), including oncosis (Mura et al., 2007), cathepsin-dependent cell death (Zheng et al., 2000) and autophagy (Chen et al., 2008). These novel cell death mechanisms are more pro-inflammatory than classical caspase-dependent apoptosis.

As a new player in the field, the use of nanoparticles in medicine should not be discouraged by receiving a yellow card; rather, it is simply a warning that care has to be taken. We have to develop a better understanding of the mechanisms of injury caused by nanoparticles, which will then allow us to devise strategies that make use of the positive features of nanoparticles whereas mitigating the detrimental consequences. There are many examples of therapies that initially ‘received yellow cards’ but after appropriate research, went on to improve the care of critically ill patients. Adequate oxygenation is essential for patients with ALI; however, oxygen toxicity due to the use of very high concentrations of inhaled oxygen (FIO₂) was recognized in the 1960s, leading to the use of lower FIO₂ and improved outcomes. Mechanical ventilation is another life-saving therapy for patients with ALI; but the use of large tidal volumes can worsen lung injury (Tremblay et al., 1997). Development of lung protective strategies based on animal and human research led to a large multi-center, randomized clinical trial that demonstrated that ventilation with lower tidal volumes could markedly reduce mortality of patients with ALI (2000).

Studies like the one by Li and colleagues are important in helping us to identify which therapeutic approaches require ‘yellow cards’. Most importantly, by identifying the underlying molecular pathways, studies such as this one will hopefully help us find novel approaches for minimizing the side effects of nanoparticles so that they can fulfill their promise as exciting materials for diagnosis and therapeutics.

References