

want to be resuscitated? We are shifting full resuscitation from the hospital, where do-not-resuscitate orders are considered, into the community. Do people need to be encouraged to wear an advance directive (perhaps on a medical bracelet or necklace) and should the volunteer resuscitator look for this before attempting resuscitation or defibrillation? To maximise benefit, it seems reasonable to combine initial defibrillation initiatives with previous and promising neuroprotective measures, especially hypothermia, in the field and in the hospital.<sup>6-9</sup>

Further work and thought are needed before widespread adoption of out-of-hospital defibrillation. We require better studies of outcomes and strategies that optimise results by integrating early recognition and out-of-hospital defibrillation; efficient transfer to a facility; and full supportive measures, including hypothermia. Although such studies are difficult to do, they are needed to justify the monumental change in infrastructure necessary to make these resources widely available. However, the studies have the potential to easily demonstrate benefit, since, at best, only 5% of current cardiac-arrest victims survive to hospital discharge and neurological morbidity is prevalent in survivors. We must address societal, ethical, and practical considerations before there is a major policy change. Input from society, government, and health-care professionals is needed. At present

we are just past proof of concept but well short of proof of benefit.

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## Predicting individual outcomes in hepatocellular carcinoma

Hepatocellular carcinoma is one of the most common cancers and its incidence is increasing in many countries because of the spread of hepatitis B and C viruses.<sup>1,2</sup> Despite recent surgical advances, about half the patients with hepatocellular carcinoma die of the disease after hepatectomy.<sup>3</sup> We must correctly classify recurrence and non-recurrence of this cancer to find a more effective therapy and to improve the poor outcome of this disease. Much effort has been devoted to identify specific gene alterations associated with hepatocellular carcinoma.<sup>3,4</sup> However, no predictive system has yet been found to be superior to morphological classification. This situation frustrates both hepatologists and pathologists.<sup>5</sup>

Recently, Ju-Seog Lee and colleagues<sup>6</sup> reported a 406-gene signature obtained from DNA microarray technology that was predictive of survival of patients with hepatocellular carcinoma, and provided new insights into the molecular basis of the disease. The researchers' goal was to create a uniform system to accurately predict survival. However, can only one microarray-based system predict individual outcomes for patients with hepatocellular carcinoma? Unfortunately, the answer is probably no, even in Lee and colleagues' update. There are two major issues to resolve before we can predict individual outcomes for patients with this disease.

The first issue is the sample label. The supervised-learning method requires a sample label to construct a predictor.<sup>7</sup> In a supervised-learning approach, we and other investigators<sup>7-9</sup> have developed microarray-based assays to predict early intrahepatic recurrence of hepatocellular carcinoma after curative surgery or intrahepatic spread of the disease at the time of surgery. Comparing the four studies (table),<sup>6-9</sup> we believe that the sample label used might be the most important factor in supervised learning. Lee and colleagues<sup>6</sup> used the sample label "two HCC subclasses of long- and short-term survivals" in the training samples. However, it is unclear how they followed the patients up and how they selected adjuvant therapy. The death of some patients with only short-term survival might be attributable to metastases in distant organs, or in remnant liver, or in both. Thus we need exact sample labels that correspond to various modes of metastases of hepatocellular carcinoma to make accurate predictions.

Lee and colleagues' sample label (ie, overall survival of patients with hepatocellular carcinoma)<sup>6</sup> could also be affected by both the cancer itself and complications attributable to liver impairment induced by hepatitis B or C viruses. Half their patients had cirrhosis of the liver with potential for de-novo hepatocellular carcinoma. It would be impossible to predict death because of de-novo hepatocellular carcinoma on the basis of the gene signature of the primary hepatocel-

	Lee et al <sup>6</sup>	Iizuka et al <sup>7</sup>	Ye et al <sup>8</sup>	Kurokawa et al <sup>9</sup>
Aim	Prediction of overall survival of patient after partial hepatectomy	Prediction of early IHR within 1 year after curative hepatectomy	Classification of HCC with intrahepatic metastasis and that without intrahepatic metastasis at surgery	Prediction of early IHR within 2 years after curative hepatectomy
Sample label*	HCCs from patients with long- and short-term survival	HCC with early IHR and HCC with non-recurrence	HCC with IM and HCC without IM at surgery	HCC with early IHR and HCC with non-recurrence
Microarray used	Oligonucleotide array representing 21 329 genes	Oligonucleotide array representing 7070 genes	cDNA microarray representing 9180 genes	PCR-based array representing 3072 genes
Sample	Total 91 primary HCC samples from 90 patients; 56 (62%) HBV-positive patients; 45 (50%) patients with liver cirrhosis	Total 60 primary HCC samples from 60 patients; 40 (67%) HCV-positive; 36 (60%) patients with liver cirrhosis	Total 40 primary HCC samples from 40 patients; all HBV-positive HCCs	Total 100 primary HCC samples from 100 patients; 41 (68%) HCV-positive patients in training set; 33 (55%) patients with liver cirrhosis in training set
Study design	Predictive system construction with 45 training samples Evaluation of predictive system on 44 independent samples	Predictive system construction with 33 training samples Evaluation of predictive system on 27 independent samples	Predictive system construction with 20 training samples Evaluation of predictive system on 20 independent samples	Predictive system construction with 60 training samples Evaluation of predictive system on 40 independent samples
Algorithm used	Five-type predictors with 406 genes	Fisher linear classifier with 12 genes	Compound covariate predictor with 153 genes	Weighted voting algorithm with 20 genes
Predictive accuracy	Accurate prediction of long-term and short-term survivors by $p=0.008-0.036$	25 of 27 (93%)	17 of 20 (85%)	29 of 40 (73%)
Characteristics of genes used for prediction	Up-regulation of cell-growth-related genes in HCC of patients with shorter survival	Down-regulation of immune response-related genes in HCC with early IHR	Osteopontin overexpression in metastatic HCC	Up-regulation of E-cadherin and down-regulation of immune response-related genes such as HLA-A and HLA-B in HCC

HBV=hepatitis B virus; HCV=hepatitis C virus; IHR=intrahepatic recurrence; IM=intrahepatic metastasis (intrahepatic spread at surgery). \* Supervisor provides label for each sample by which we can construct predictor in supervised learning manner. In our study, we highlighted gene selection rather than classifier design. Our successful result comes from novel gene selection method that can cope with variability of training samples.

**Table: Four transcriptome-based predictors of metastatic potential of hepatocellular carcinoma**

lular carcinoma. In our follow-up,<sup>7</sup> the sample label “early IHR within 1 year after surgery” did not allow accurate prediction of late intrahepatic recurrence attributable to de-novo hepatocellular carcinoma or distant recurrence (data not shown). Several de-novo hepatocellular carcinomas might be included in the sample label “early IHR within 2 years”,<sup>9</sup> decreasing the prediction’s accuracy.<sup>10</sup> Thus the recurrence of hepatocellular carcinoma is more complicated than that of other cancers, suggesting that molecular profiling with the use of a single sample label might be limited in predicting individual outcomes in patients with hepatocellular carcinoma. In our opinion, combining a system for clinical staging of liver dysfunction<sup>2</sup> and several systems based on molecular profiling of primary hepatocellular carcinoma to understand metastatic potential will enable us to make such predictions.

Another problem with predicting individual outcomes in patients with hepatocellular carcinoma is accounting for patients’ different backgrounds. The four studies<sup>6-9</sup> used heterogeneous cohorts, various arrays, and distinct algorithms (table). There were two cohorts with different virology: patients with hepatocellular carcinoma predominantly related to hepatitis B virus<sup>6,8</sup> and those with hepatocellular carcinoma predominantly related to hepatitis C virus.<sup>7,9</sup> A previous molecular profiling study<sup>11</sup> showed that a difference in hepatitis virus infection can greatly affect gene-expression patterns of primary hepatocellular carcinoma. These studies<sup>6-9</sup> also had patients with different racial and geographic origins. Larger studies are needed to identify common metastasis-related genes among patients with hepatocellular carcinoma who have different backgrounds, as acknowledged by Lee and colleagues.<sup>6</sup>

Predicting individual outcomes for hepatocellular carcinoma patients after they are treated remains a challenge. To overcome this dilemma, first we must identify the sample labels needed to construct accurate predictors. Second, we must consider patients’ backgrounds. The work of Lee and colleagues,<sup>6</sup> with a larger microarray and larger sample sizes, might be the research that is closest to addressing these two issues.

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## Potential neurovirulence of common cold virus

An elegant recent study by Thomas Dufresne and Matthias Gromeier<sup>1</sup> suggests that a causative agent of the common cold, coxsackievirus A21 (CAV21), is potentially neurovirulent and could, under the right circumstances, cause a poliomyelitis-like illness. CAV21 and poliovirus are members of the enterovirus genus (family *Picornaviridae*) and show remarkable genetic similarity.<sup>2</sup> However, CAV21 causes upper respiratory-tract infections whereas poliovirus causes neurological disease including poliomyelitis, which clinically manifests as acute flaccid paralysis. The contrast in clinical presentation has been attributed to the different receptors used for cell invasion: intercellular adhesion molecule-1 (ICAM-1) is the receptor for CAV21 and CD155 is the receptor for poliovirus. Not surprisingly, the genomes of these two related viruses show the greatest dissimilarity in the capsid (or viral coat) region.<sup>3</sup>

Murine ICAM-1 does not support CAV21 binding and mice are not normally susceptible to infection with this virus. Dufresne and Gromeier<sup>1</sup> stably inserted the gene encoding human ICAM-1 (hICAM-1) into the genome of a mouse (which thus became transgenic), resulting in expression of hICAM-1 by the animal's cells. They then inoculated one gastrocnemius muscle of the transgenic mouse with CAV21. Acute flaccid paralysis developed in the injected muscle, but not in any other muscle. CAV21 replication, motorneuron destruction, and inflammation were detected in the ipsilateral anterior horn of the spinal cord, but viral replication was not seen in the injected muscle. The abnormalities in the spinal cord did not occur if the sciatic nerve was transected before inoculation (figure). These observations suggest that CAV21 can be pathogenic for motorneurons and that this neurovirulence is dependent on invasion of the central nervous system by retrograde transport along nerve axons.

These findings are scientifically important because they suggest that CAV21 has all the machinery required to cause a poliomyelitis-like illness, but is prevented from doing so by virtue of its receptor not being expressed at the neuromuscular junction. Dufresne and Gromeier<sup>1</sup> found low levels of hICAM-1 at the neuromuscular junction of hICAM-1 transgenic mice, but not in wild-type mice. Unfortunately, they did not report whether there is hICAM-1 expression at the human neuromuscular junction, so leaving doubt as to how the observations translate to human beings. They also found hICAM-1 expression on motorneurons in the spinal cord of transgenic mice, but no paralysis occurred when CAV21 was injected directly into the central nervous system. This apparently paradoxical observation could be explained

by a requirement for neuronal infection of a co-receptor that is expressed at the neuromuscular junction, but not by neurons in the central nervous system. A role has been found for decay accelerating factor as a coreceptor for CAV21 attachment to human cell lines<sup>4</sup> and, indeed, decay accelerating factor is expressed at the neuromuscular junction,<sup>5</sup> but could not be found on neurons in the central nervous system.<sup>6</sup>

2004 sees the 50<sup>th</sup> anniversary of Enders, Weller, and Robbins receiving the Nobel Prize in Medicine for culturing poliovirus,<sup>7</sup> and the year coincides with the final stages of the Global Polio Eradication Programme. How clinically significant is the discovery of neurovirulent potential for CAV21 from a public-health perspective and should it concern us? Dufresne and Gromeier<sup>1</sup> chose to refer to the acute flaccid paralysis observed with CAV21 infection in hICAM-1-transgenic mice as "poliomyelitis". In line with WHO definitions, we think this term is best reserved for poliovirus and that "poliomyelitis-like illness" should be used for acute flaccid paralysis caused by other agents.<sup>8</sup> Thus poliomyelitis eradication is not directly affected by Dufresne and Gromeier's findings, but their results increase our concern that the global public-health burden of acute flaccid paralysis will not disappear with the eradication of poliovirus.

Several reasons suggest that CAV21 itself is unlikely to be a clinically significant cause of poliomyelitis-like illness in human beings. CAV21 infection in the hICAM-1 transgenic mice is site-restricted and much less aggressive than poliomyelitis in human beings. Paralysis only occurred in the hICAM-1 transgenic mice via the intramuscular route and not with intravenous, intranasal, or central nervous system routes, and paralysis remained localised to the injected muscle. This observation is analogous to "provocation poliomyelitis" where skeletal muscle injury predisposes an individual to poliomyelitis from concurrent poliovirus infection.<sup>9</sup> Unlike CAV21 in hICAM-1 transgenic mice, poliovirus can spread to the central nervous system directly in the context of viraemia, as well as via retrograde axonal transport. In addition, poliovirus usually enters human beings via the oral route and not by the direct intramuscular route. Dufresne and Gromeier<sup>1</sup> did not report the result of oral administration of CAV21 in hICAM-1 transgenic mice.

What could account for the difference in severity between CAV21-induced poliomyelitis-like illness and poliomyelitis? The most likely answer is differences in binding of viral capsid with either hICAM-1 or CD155 and the tissue distribution of these receptors and any relevant co-receptors, such as decay accelerating factor. CAV21 could present more of a threat to