国际工程科技发展战略高端论坛

International Top-level Forum on Engineering Science and Technology Development Strategy

中国工程院
CHINESE ACADEMY OF ENGINEERING

病毒性肝炎和肝病—20 年展望

BINGDUXING GANYAN HE GANBING—20 NIAN ZHANWANG

VIRAL HEPATITIS AND LIVER DISEASES—TWO DECADES AHEAD

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国际工程科技发展战略高端论坛：病毒性肝炎和肝病—20 年展望与会专家合影（2012. 6. 22）

主报告会场

内容提要

病毒性肝炎、肝硬化、肝癌是严重影响我国人民健康的重要疾病。为了提高对这些疾病的诊治水平及展望今后防治的前景，根据2012年举办的国际工程科技发展战略高端论坛：病毒性肝炎和肝病—20年展望，集十余位美、德、英、日及我国的顶尖专家们的报告内容，本书进行了编辑与加工。全书涵盖了病毒性肝炎流行病学特点、免疫应答与新疫苗研发、抗病毒治疗、肝癌诊治新方向及今后治疗肝病的新模式等。书中既有概括性的内容综述，也提供了各位专家的报告内容及相关幻灯片。为了使更多读者能直接阅读原文，全书提供了中、英两种文字内容。本书是中国工程院国际工程科技发展战略高端论坛系列丛书之一，是一本有重要参考价值的专著，可供感染病及肿瘤科临床医师、流行病学工作者、疫苗研发和基础医学研究者及研究生参阅。

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复旦大学上海医学院

一、论坛背景

病毒性肝炎、肝硬化、肝癌是一组严重影响我国人民健康的重要疾病。迄今，病毒性肝炎可分别由甲、乙、丙、丁及戊型五种病毒引起。其中甲型和戊型肝炎主要由胃肠道转播，即可由污染食物、水源所传播，一般不会引起慢性肝炎、肝硬化或肝癌。虽然甲型肝炎病毒1988年曾在我国上海等地区引起过暴发流行，但自从我国研发成功并广泛应用预防性甲型肝炎疫苗，甲型肝炎发病率已显著下降。一般戊型肝炎也不会引起慢性肝炎，但近年在一些免疫低下的患者中发现戊型肝炎病毒可长期持续存在，因而也有个别因戊型肝炎引起的慢性肝炎，但未发现戊型肝炎可引起肝癌。乙型肝炎及丙型肝炎均主要由血源传播，同时也可由母婴及性传播。丁型肝炎病毒需与乙型肝炎病毒共同复制与传播，不单独复制，在我国同时感染乙型、丁型肝炎的患者很少。目前在我国，慢性乙型肝炎及其相关的肝硬化及肝癌是最严重的卫生与健康问题，丙型肝炎及相关的肝病及肝癌也占有重要地位。据中国卫生部统计，2011年我国（除港、澳、台外）上报病毒性肝炎的发病率为102.34/10万，发病例数达1,372,344例，居28类应报告传染病之首，而其中乙型肝炎又是各类病毒性肝炎之首。据中国卫生部公布的恶性肿瘤发病与死亡种类的数据，男性中肝癌占第二位，在女性中占第三位。由此可见乙型及丙型肝炎、肝癌对我国人民危害的严重性。

虽然早在1992年我国已开展了对新生婴儿乙肝疫苗的预防接种，在2002年又将新生儿乙肝疫苗预防接种纳入免疫规划，2005年新生儿乙肝疫苗预防接种由国家全额支付所需费用，据2006年乙型肝炎血清流行病学调查，全国乙型肝炎病毒表面抗原携带者已降至7.18%，但仍有3%左右人群对现有乙型肝炎疫苗无应答或低应答。据估算，目前我国乙型肝炎病毒携带者约有9300万人。丙型肝

炎病毒感染者约1000万例，这两种病毒感染均可导致肝硬化及肝癌，迄今乙型肝炎尚无有效的根治方法。因此，我国“十一五”及“十二五”科技重大传染病专项中，均将乙型肝炎包括肝癌作为重点研究并控制的疾病。其中特别列出需显著降低乙型肝炎的感染率与病死率。

“科学技术是第一生产力”。通过集国内外专家的智慧，本次高端论坛的目标是，通过共同交流与展望，高瞻远瞩，指出在今后20年对病毒性肝炎及肝病、肝癌研究的方向与策略，从而逐步达到控制并可能最终消灭乙型肝炎的目的。

二、论坛整体情况

本次高端论坛于2012年6月22日在上海国际会议中心举行，选择论坛的时间为在我国上海召开的第十四届国际肝炎肝病大会（ISVHLD2012）前一天。鉴于该大会是自1972年首次召开、40年来每3年举行一次的全球大会，参会的专家层次高，覆盖面广，因而具有很强的国际影响力。在此基础上召开高端论坛将可汇集多国及国内专家到会，可全面、深入地探讨及展望今后20年病毒性肝炎、肝癌存在的问题，从宏观上提出整体策略与制订解决方案。

论坛由中国工程院主办，复旦大学上海医学院、上海市中国工程院院士咨询与学术活动中心、中国工程院医药卫生学部共同承办。由中国工程院副院长樊代明院士主持，中国工程院院长周济院士及工程院机关部门10人到会。周济院长指出，举办国际工程科技发展战略高端论坛旨在为世界顶级专家搭建高水平高层次的国际交流平台，通过宏观性、战略性、前瞻性的研究，探讨工程科技领域的宏观和战略问题。病毒性肝炎和肝癌作为一类世界范围内流行的疾病，严重威胁着人类的健康。加强肝炎和肝病的研究力度，事关经济发展大局和社会和谐稳定。生物技术的发展为肝炎和肝病研究带来了新的机遇和挑战。在这样一个重要的转折阶段，对未来20年肝炎和肝癌研究发展前景进行研讨，提出宏观发展战略具有重要意义。

会议根据专题，凝练出以下几方面进行了研讨：病毒性肝炎及肝病的发展趋势及防治重点；基础与应用研究的方向；个体化治疗与方针；如何将治疗重点前移。针对这些专题，在报告人做简短发言后，到会专家参与讨论者共32人。会上，美国贝勒医学院 Blaine Hollinger 教授、日本东京大学 Masao Omata 教授、加拿大亚伯达大学 Michael Houghton 教授、德国汉诺威医学院 Michael P. Manns 教授、德国埃森大学病毒所 Michael Roggendorf 教授、美国德雷克塞尔大学医学院 Tim Block 教授、复旦大学附属中山医院汤钊猷院士、第二军医大学附属东方肝胆外科医院王红阳院士、南方医科大学附属南方医院感染内科侯金林教授、中国疾病预

防控制中心病毒病预防控制所阮力研究员、中国疾病预防控制中心王宇主任等国内外专家围绕新型疫苗与免疫、病毒与变异、临床研究与新的治疗手段、新药研发、肝癌控制的新趋势等主题作了专题发言，杨胜利院士、郑树森院士、庄辉院士等与会专家围绕报告内容展开了研讨，为提高我国病毒性肝炎及肝病的临床及基础研究与防治水平献计献策。会议共有 6 名中国工程院院士（闻玉梅、杨胜利、庄辉、郑树森、汤钊猷、王红阳），及来自 5 个国家的 12 位外国专家（其中美国科学院院士 2 人），以及来自我国 6 个省市的专家教授 14 人参加会议。

会议结束前由周济院长向各报告人颁发了发言证书，并与全体与会专家共同合影留念。

三、高端论坛专家发言及研讨内容

（一）疫苗研发仍是控制病毒性肝炎及肝癌的主要工具

虽然灭活与减毒甲型肝炎疫苗已在我国广泛应用，并已显示良好的效果，但研发疫苗的分子减毒，组建基于病毒样颗粒的新疫苗，以及发展多价减毒与多价亚单位疫苗等仍是今后需要发展的方向。乙型肝炎疫苗的预防效果十分显著。2006 年全国乙型肝炎血清流行病学调查显示，我国一般人群的乙型肝炎病毒表面抗原携带率已由 9.75% 降至 7.18%。其中 1～4 岁儿童已降至 0.96%。但是仍有乙型肝炎病毒表面抗原及 E 抗原双阳性母亲分娩的婴儿，约 3% 未能被疫苗加乙型肝炎免疫球蛋白联合免疫所保护。由于婴幼儿期感染乙型肝炎病毒者较成年后感染者易发展成慢性，且发展成肝癌的几率也较高，因此，需要研发针对现有乙型肝炎疫苗低反应/无反应婴儿的新型预防性疫苗。目前，虽然对丙型肝炎患者已有有效的药物治疗方案，但因治疗药物价格昂贵及可出现不良反应等因素，仍有研发丙型肝炎预防性疫苗的需要。国际现有丙型肝炎预防性疫苗临床研究的项目值得我们参考或合作。我国自行研发的戊肝疫苗已获准上市生产，但大规模应用的对象选择及预防效果仍有待分析研究。

（二）针对肝癌的基础与综合性研究将是今后的重中之重

迄今经过综合治疗，虽然肝癌的 5 年生存率已由 7.4% 上升至 44%，但因乙型肝炎或丙型肝炎病毒感染所致的肝癌今后 20 年仍将继续危害人民健康，因此有效地早期发现与治疗肝癌是关键。面对众多生物标记物（biomarker）的研究与应用，如何正确地、因人而异地综合选用几种或一组生物标记以达到早诊早治，将是重点。其中 GP73 的价值及不同岩藻糖基化的生物标记物等都需要根据不同

患者、不同阶段进行深入、有对照的应用研究与分析。专家们强调，慢性未控制的炎症是肝癌发生发展的重要因素，因此要尽早控制炎症，减少炎症细胞浸润及伴有的细胞因子、趋化因子的不良作用。代谢因素在肝癌中的作用也不可忽视。糖尿病、肥胖、大量酒精摄入以及黄曲霉素污染均与肝癌发生相关，需予以重视。日本教授介绍了该国应用第二代测序技术对大量肝癌组织深度测序的初步成果与应用前景，提出应用这些技术将可对不同个体间、不同肝癌组织间的共性与特性及差异做大量组学的研究。根据组学研究获得的大量数据将可研发出新的分子靶类与药物。专家们建议要建立对肝癌进行“消灭 + 改造”的综合治疗思路，今后研究重点可聚集在肿瘤的转移与复发，而对肿瘤起始细胞的深入研究是阐明肝癌分子机制，控制转移与复发的主要环节。

（三）探讨并实施个体化治疗是今后治疗病毒性肝炎的方向

虽然目前针对乙型与丙型肝炎的治疗均已有干扰素及核苷类药物，但是为了提高疗效，降低成本，探讨与采用有效的个体化治疗是今后病毒性肝炎临床研究的主要方向。为此，需要先建立更能代表患者不同疾病过程的动物模型进行实验性探索。在研发新的抗病毒药物的同时要大力发展新的治疗技术与路线，其中可包括应用多种免疫治疗/免疫调节剂、激活固有免疫的制品如针对 Toll 样受体的制剂、激活特异性免疫的治疗性疫苗、用腺病毒等导入的基因治疗，以及研发能有效降低乙型肝炎病毒表面抗原水平的新制品等。在研发中要防止只研究机理，而忽视各种方法的可行性。此外在考虑个体化治疗时，要避免“多种药物或措施一起用”、不计较成本的倾向。鉴于个体的反应差异，在采取一种或多种综合治疗时，应特别注意研究并观察患者的早期应答指标，从而可更完善地制订及选择对患者的个体治疗方案。

（四）建立多中心大型网络，落实资源共享，提升医疗服务水平

德国专家全面、细致地介绍了德国在肝炎肝病方面有计划、有组织地组建大型网络的过程、组织架构与管理措施。其中包括他们如何科学、统一标准地收集欧洲数万及数十万乙型及丙型肝炎患者的临床及实验室的资料，并加以管理。参加网络的各单位可以互相交流，并定期从网络资料库中获取材料，共同作大样本的分析。专家们提出今后医学的发展必然要走大样本、资源共享、大协作研发的道路。单一中心的研究体制必然会由多中心研究体制所取代。作为医学，要为患者服务好，必须认真遵循医学规律，以大量服务对象为资源，寻找出疾病发展趋势及诊治效果，同时兼顾治疗方法的社会与经济效益等，整合资源才能提高服务效率。

（五）改革现行医疗及教育体制将显著影响肝炎及肝癌的发展

美国学者全面预测了20年后肝病学家的任务，从3个方面提出了方向：（1）加强对基层医务工作者的培养，发行简易工作手册。要让基层医务工作者了解如何在社区早期发现感染者，及时给予治疗将大大减少晚期出现并发症的患者，降低治疗成本。（2）发展非课堂式教学，充分运用网络，视频教学，引导学生自学为主，不单纯依赖课堂教学，争取学生们成为主动型的求学者。（3）医学与信息科学同步发展。肝病医师将改变仅利用现有技术手段的诊治方式，而是应学习、了解智能手机、平面电脑、云计算等新技术并能及时应用新技术的学者。因此加强医疗体制改革，重视社区医生的培养与提高，运用网络、视频技术，改进教学方式将可更好地预防及治疗肝病。

高端论坛提出了有前瞻性的新思路、新观点、新策略，达到了从顶层、宏观水平思考及设计的目的。参会专家们希望3～5年后再次召开类似会议，以检验所提出的预测与建议的实际价值，并可对有关问题进一步研讨。

闻玉梅

复旦大学病原微生物所教授，教育部/微生物分子病毒学重点实验室教授

毕业于上海第一医学院。1980年赴英国伦敦大学卫生与热带病研究所进修肝炎病毒，1981－1982年在美国NIH，NIAID肝炎研究室做Fogarty访问学者。主要研究方向为乙肝病毒分子生物学与免疫学及微生物功能基因组学。是乙肝治疗性疫苗的开创者。在国内外已发表240篇论文，主编现代微生物学、英文版的中国病毒性肝炎—问题与对策，及微生物与感染等专著。

1999年入选中国工程院院士。曾任《Archives on Virology》执行编委，现为《Emerging Microbes and Infections》杂志共同主编。2006年亚太医学微生物学会授予医学病毒学特殊荣誉奖，2009年被德国Duisburg and Essen大学授予荣誉博士。

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第三部分

主题报告及报告人简介

未来控制肝癌的展望

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回顾肝癌治疗的历史,其平均生存期已由原来的 9 个月延长至 90 个月。外科手术治疗加上射频术后治疗成功地延长了病人的生命(图 1,2)。

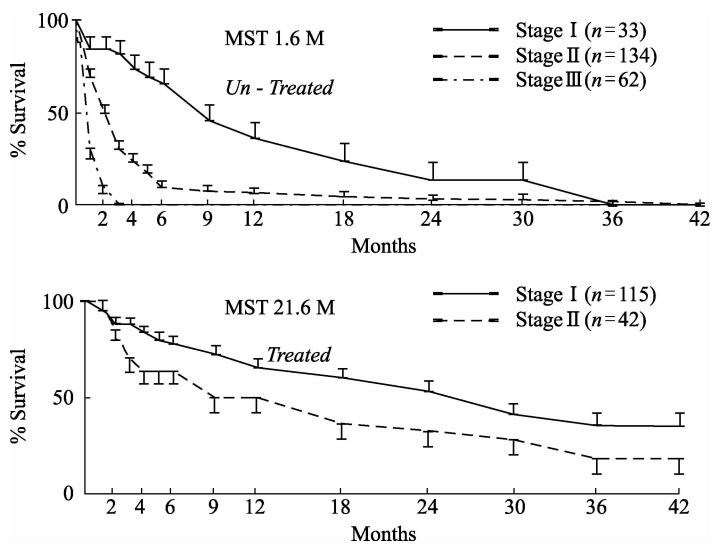

图 1　不同期肝癌患者治疗与未治疗的生存百分率(MST 9 Months)

(Okuda. Natural History of HCC; 850 cases Cancer 1985,56:918 -)

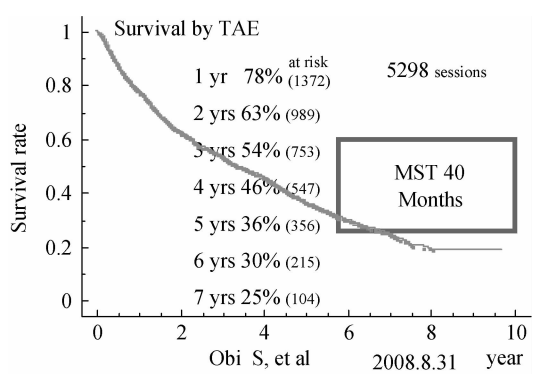

图 2　中位生存期

乙肝疫苗预防注射显著降低了乙肝感染率并预防了肝癌在大多数国家中的发生。此外,用抗病毒治疗也可降低病毒所致的肝癌发生率。Entecavir 和 Tenofovir 是抑制乙肝病毒复制的药品。近年来,有资料显示,用抗病毒治疗可降低肝纤维化的发生。通过抗乙肝病毒治疗,可每年降低 0.26 metavir score,与丙肝病人清除 HCV 的回归率 0.28 相近。因此,减少肝纤维化肯定有利于肝癌的治疗,为了更有效地改善肝纤维化的预后,加入抗病毒治疗可以减少具有肝硬化基础患者的复发率。但是用抗病毒治疗乙肝及丙肝患者也有局限性,如干扰素的副作用尤需注意。由于丙肝抗病毒治疗药物的进展,这一问题已得到显著改变。至今许多抗病毒药仅有很少的副反应,在有高 Child-pugh 分数的病人中仍可应用。这些药物的应用将改变我们过去在治疗肝癌中遇到的难处。

最后,治疗肝癌还需要发展抗肿瘤的药物,近来,特别应重视分子靶类药物的应用,但至今 Sorafenib 的疗效仅能延长患者生存期 3 个月(图 3)。

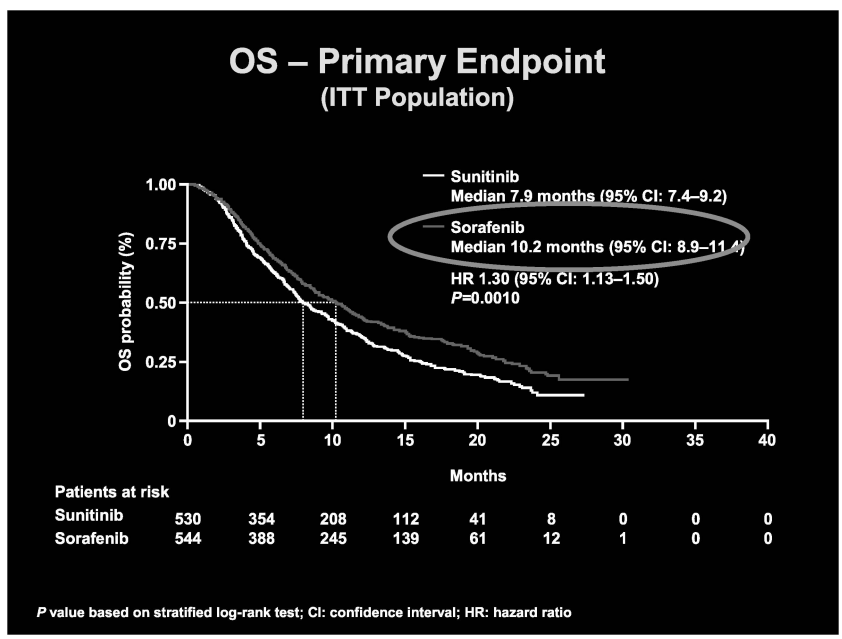

图 3　Sorafenib 治疗的患者平均生存期

为了进一步发展这一治疗途径,应该对肝癌细胞分析其基因结构与表达谱[1]。我们研究了肝癌的基因组学,应用下一代的测序,我们将开始从基因组学水平解析个体间及各肿瘤组织间的多样性。一个肝癌测序的新纪元即将开始(图 4,5)。

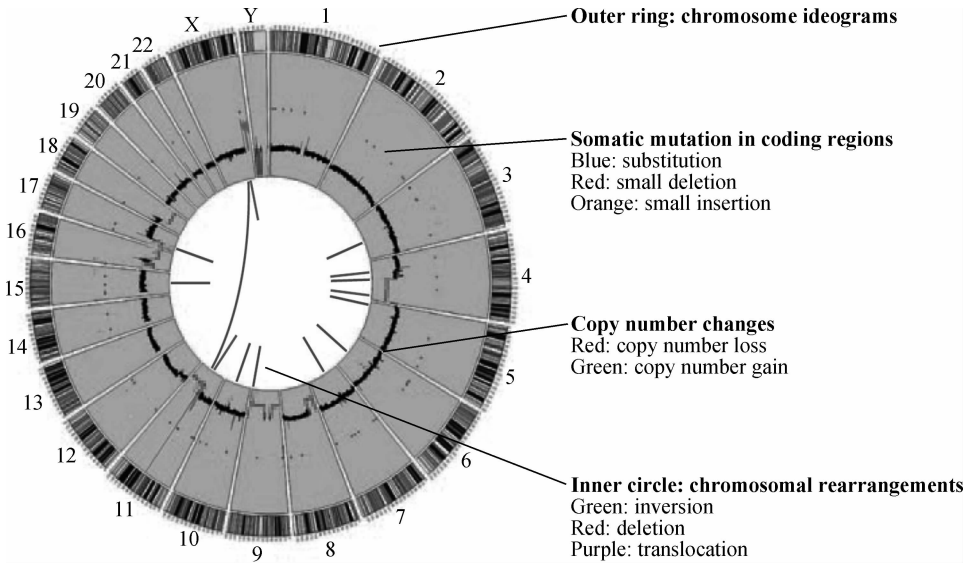

图 4　体细胞水平获得的肝癌全基因组变化图

[Permission obtained from Nature Publishing Group© Totoki et al. Nat Genet 43, 464—469(2011)]

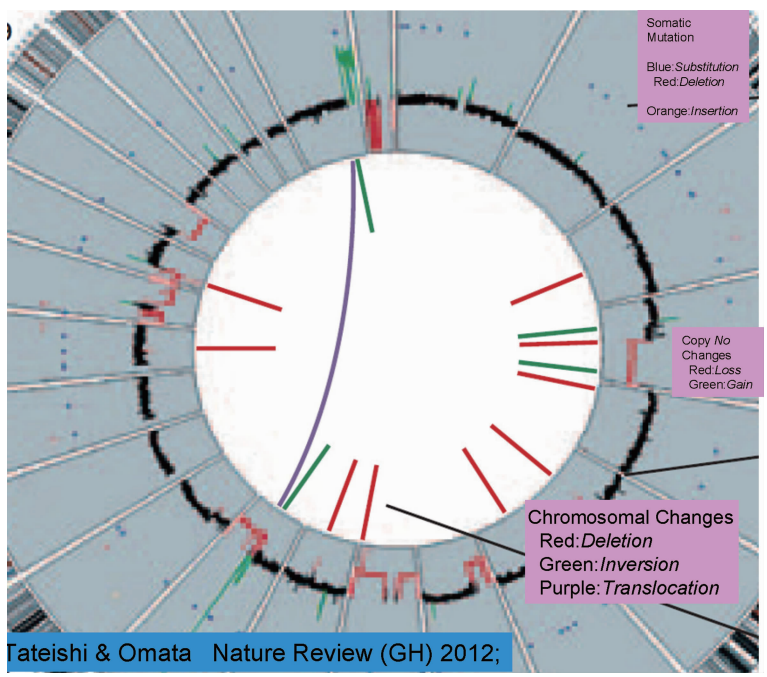

图 5　体细胞水平获得的肝癌全基因组变化图

对比 1979 年及 2012 年的测序水平,可以预测,通过本研究将可获得大量的数据,并将可能确定启动子的突变,从而可以提供新的分子药物(图 6)。

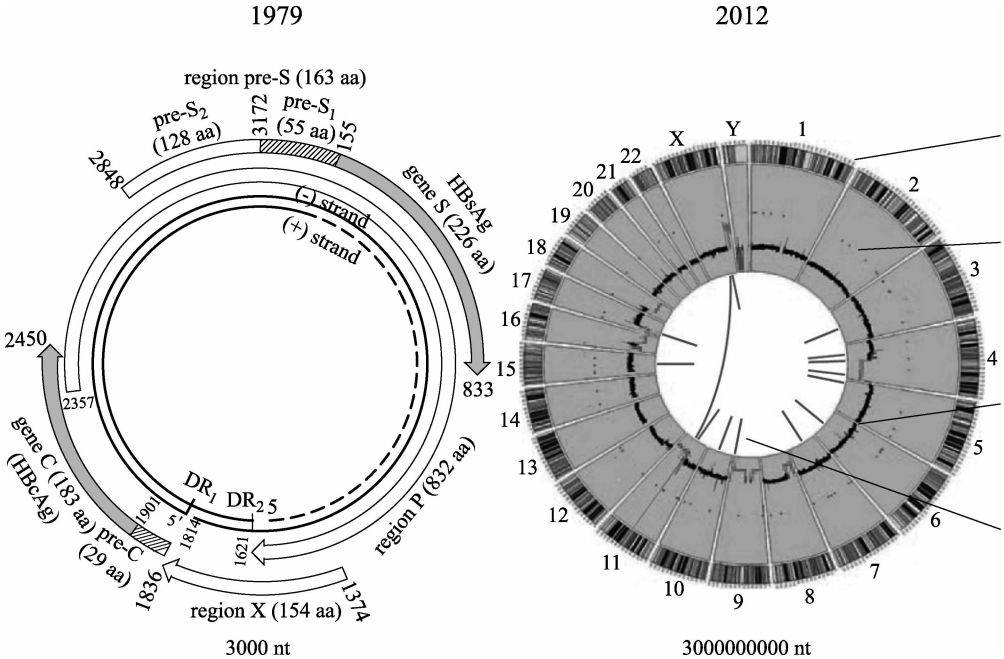

图 6　1979 年及 2012 年可以获得的核苷酸序列数

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Masao Omata 教授目前为《Hepatology International》杂志的共同主编。1970 年毕业于日本千叶大学,毕业后就职于千叶大学第一内科学系。在日本接受 3 年培训后,先后在美国耶鲁大学和南加州大学继续进修,并接受了 Gerald Klatskin 教

授和 Robert L. Peters 教授的指导。

1979 年 Omata 教授返回日本千叶大学,在千叶大学工作期间他将分子生物技术引入临床胃肠病学,先后发表论文 90 余篇。1985 年和 1991 年在《N Engl J Med》杂志发表两篇人与鸭乙肝病毒分子生物学方面的论文。他最早报道了 pre-core 变异和急性肝炎的相关性,以及干扰素可高效消除急性丙肝。并且提示超螺旋或乙肝病毒的 CCC 基因是引起人抗病毒治疗抗药性的关键分子。

1992 年 Omata 教授到东京大学第二内科学系担任系主任,这是东京大学史上第一次聘请非东大毕业生担任它的医学院系的负责人。此后由于院系调整,Omata 教授于 1997 年担任东京大学胃肠病学系主任。

2009 年 Omata 教授成为山梨县立中央医院和 Kita 医院两所医院的院长。在东大任职的 19 年间,Omata 教授在胃肠病和肝脏病学方面先后发表基础和临床英文论文 941 篇。他还是日本第 40 届肝脏病年会、亚太肝病学会年会(2006—2007)、第 50 届日本胃肠病学会议(2008)的大会主席。目前他是《Hepatology International》(亚太肝病学会的官方杂志)杂志的主编,亚太肝病学会日本秘书处主任。

GP73 和岩藻糖基化肝癌生物标记的起落和重新崛起

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肝癌的早期检测是取得良好预后的最佳机会。不幸的是,肝癌的非侵袭性的血清学标记价值有限,而常用的标记甲胎蛋白(alpha fetoprotein, AFP),仅在 50% 的肝癌病人中为阳性。我们和其他研究团队已经用糖蛋白组学的方法发现了一些新的标记物。我们报道的一个高尔基体定位的蛋白 GP73,又称 Golph 2 或 GOLM1,在诊断为肝癌病人的血液中明显增高。在多数研究中表明,GP73 在鉴别诊断肝硬化和肝癌方面的能力远胜于 AFP。然而,也有一些报道发现 GP73 逊于 AFP。本文总结了最近的进展,解释 GP73 和其他生物标记物在识别对照和疾病受试者过程中的不同表现。简而言之,我们发现了相对于单一检测总 GP73,GP73 各种特异的糖基化形式对肿瘤的检测更有选择性区别的价值。而且,GP73 在无肝病的人群中很少有增高现象,但是在肝硬化的病人中它经常是增高的。因此,通过结合某些临床特征,GP73 和其他生物标记对肿瘤的预测价值将会极大改进。

肝细胞癌(Hepatocellular cellular carcinoma, HCC)在世界癌症致死因素中排行第三,每年导致将近一百万人死亡[1-3]。在中国的某些地区,它是年轻男性最常见的致死因素[1,2,4-6]。现在,在美国,它是少有的几个发病率呈上升的癌症之一[1,2,4-7]。

早期检查可以极大地改善预后[7-9]。血清蛋白质类或是能促进对疾病风险的评估,或是可作为早期检查流程中的一部分。在肿瘤很小且可治疗时,它们的意义极其重要,能使病人去做成像图诊断和癌症治疗。通用的非侵袭性的生物标

记 AFP 和其岩藻糖基化形式 L3，甚至一些新的生物标记，例如 DCP，检测价值有限，在所有病例中大约仅有 30% ~70% 癌症可以被检测到[10]。在所有肝癌病例中，约占 25% ~50% AFP 阴性的肝癌里，AFP 和 L3 也是没有检测价值的[11]。

我们已经发现和报道了，在诊断为肝硬化合并肝癌的病人中，通常定位于高尔基体膜的蛋白 GP73 出现在血液中，而且该蛋白在血清中的水平可能在肝癌检测中很有价值[12,13]。现在已知的几个独立的报道普遍地验证了我们的结果，都认为 GP73 是肝癌[14-18]和肝病[19-22]检测中的一个可用的生物标记。

最值得注意的是，最近的一次在超过 4000 人（其中 800 人为肝癌病人，600 人为肝硬化病人）的盲测中，显示 GP73 比 AFP 具有更强的敏感性和特异性[22]。

实际上，虽然 GP73 在肾癌[23]和前列腺癌[19,24]病人的精液中也增高，然而却高度选择性地在肝癌病人血液中增高[25]。而且，尽管一些报道显示，GP73 与肝癌和肝病发展相关，但血清中检测阳性并不比 AFP 能提供更多的价值[20,26]。来自我们实验室的几项研究提供了一些例子，相对于 AFP，在区分肝硬化和肝癌样本中，在何种情况下，GP73 和岩藻糖基化的急性期蛋白表现更好，在何种情况下，表现比较差。总结列于表 1 中。

表 1　不同研究报道的生物标记物在肝硬化与肝癌的差别

	标记物[1]					
	对照/HCV/HBV/Cirr/HCC(n)[2]	AFP[3]	GP73[3]	f-hem[3]	f-kin[3] & f-AIAT	参考文献[4]
研究 1	23/0/23/0/8	N/A	0.98[5]	N/A	N/A	12
研究 2	56/0/0/152/144	0.61	0.79	N/A	N/A	13
研究 3	20/0/20/20/20	N/A	0.80	0.95		27
研究 4	00/00/00/113/164	0.83	0.89	N/A	0.79	24
研究 5	0/0/0/200/100	0.80	0.64	0.62	0.70/.55	Ref set/with IF failure samples, unpublished
研究 6	20/133/33/32/72	0.82	0.90[5]/0.89	0.95[5]/0.87	0.80	25

[1] Marker utilized in study. AFP, Alpha-feto protein; GP73, Golgi Protein 73; f-hem, fucosylated hemopexin; f-kin, fucosylated kininogen. Assays as in grant text and citations.

[2] Number of individuals in the given study with the following clinical characteristics: Controls (no evidence of liver disease); HCV, infection with HCV but no evidence of liver cirrhosis or HCC; HBV, HBV infection but no evidence of liver cirrhosis or HCC; Cirr (cirrhosis); HCC, hepatocellular carcinoma. Diagnosis and distribution of viral etiologies comprising HCC and cirrhosis as in grant text or citation.

[3] For each marker the AUROC at differentiating HCC from cirrhosis is given, excepting superscript (5); kininogen (kin), Alpha 1 antitrypsin (A1AT).

[4] Citation of publication of study.

[5] Ref sets including with rebetron failures.

[5] AUROC for HCC against all other disease categories in study.

是否,或者怎样使用这些可能的生物标记是个十分重要的问题。既然一些研究表明它们是灵敏的,那么是否要检测它们呢?既然一些研究表明它们是逊于AFP的,那么是否要丢弃它们呢?

本文研究了GP73的物理性能,同时比较了GP73作为肝癌的生物标记,在表现好和差的研究中所选取的人群的临床特征。

GP73在诊断为肝硬化的病人中增高

一系列的研究已经确认,相对于健康的受试者和肝炎患者,诊断为肝癌病人的血液中GP73的水平较高[10,13-18,20,22]。然而,正如我们在最初报道GP73作为疾病的生物标记中所观察到的,它常在肝硬化的病人中增高,而在肝癌病人中无相关的证据[12,19,28]。与这些报道一致的是,正如在分布图(图1)中所显示的,结合我们组的研究4和5(表1)中的年龄和性别匹配的样本中的数据,GP73的水平通常在肝癌病人中最高,有肝硬化的个体实际上可能比有肝炎而没有肝硬化的个体偏高,而且后两者的水平都比肝癌患者的要低。这就暗示,仅仅只有GP73的水平可能显示有病理变化,必须结合其他的信息,才能对肝癌做出可靠的危险性评估。

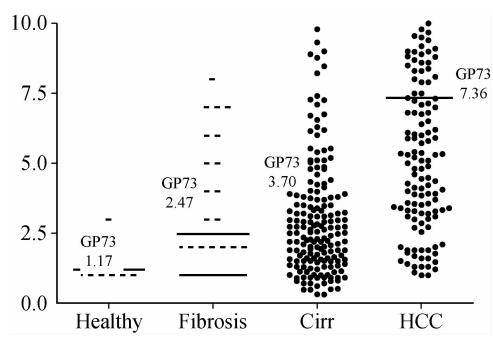

图1　GP73在肝硬化和肝癌中均增高

血清中GP73水平通过ELISA的方法确定[24]。这份数据基于两份研究的样本,包括大约250个肝硬化无肝癌受试者和150个肝硬化合并肝癌受试者,其中大约100个肝硬化和50个有肝癌者,已经接受alpha干扰素和利巴韦林(ribavirin)的治疗(文章准备中)。人群中GP73的水平作为肝病状态的反映。每组平均值用红色表示,对照组为健康受试者,其血清水平作为基准。

糖型及特异的岩藻糖的联结形式与肝癌相关

我们对血清中GP73的水平和肝癌诊断的相关性的观察,最初是基于鉴定岩

藻糖化的 GP73 与肝癌相关[12]。简而言之,我们团队和其他团队都发现来自于血液中的所有的蛋白的岩藻糖化的 N - 聚糖在肝癌病人中有明显增高的现象[12,29-31]。之后我们鉴定出一系列特异的岩藻糖化的糖蛋白,相对于肝硬化病人,它们在肝癌病人的血液中都增高,因此称这一系列蛋白为肝癌的“fucome”(岩藻糖组)[25,31]。

最主要的候选蛋白就是 GP73 和其岩藻糖化形式(尽管区分岩藻糖化形式和非岩藻糖化形式不是常规的实验)。我们认为这些蛋白是由癌症细胞直接或间接产生和过量分泌到血液中的。不能区分不同糖型的一些检测方法可能会导致这些生物标记物表现不佳。

因此我们的研究表明,这些生物标记的糖型随着疾病的状态而变化最大,而且解释了假阳性出现的原因(在没有肝癌的情况下 GP73 和 f - 急性期糖蛋白增高的病例)。例如,相对于总的糖蛋白水平而言,血清中 f - GP73 和 f - 急性期糖蛋白(例如 f-kin 和 f - A1AT),是肝癌更好的生物标记[24]。

然而,岩藻糖通过不同的联结结合于 N - 聚糖不同的位点。随后详细地分析了 A1AT 糖基化的形式(如图 2),我们最近发现 f - A1AT 的 a1,6“核心”岩藻糖化形式是肝癌最常见的选择型,而且只有这一种亚型是特异的被核心岩藻糖化[32]。

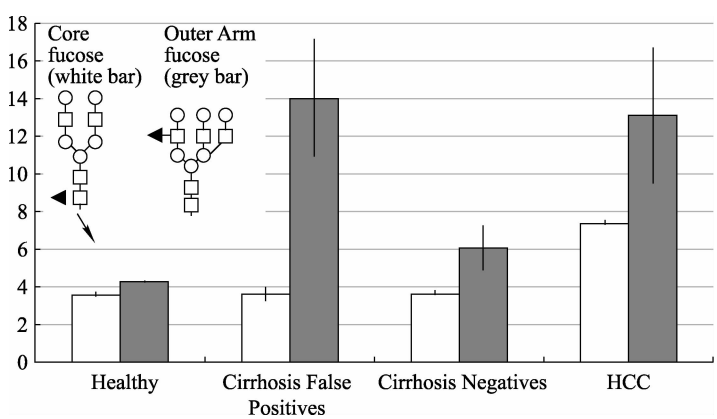

图 2　核心而不是外臂岩藻糖基化的 A1AT 水平与肝癌的诊断相关

插图:有黑色三角形的 N 联聚糖显示核心(结构在左边)或外臂(结构在右边)。柱值表明循环中核心(白色)或外臂(灰色)岩藻糖基化的 A1AT 相对水平,其中包括年龄和性别匹配的健康人、有高水平总 f - A1AT 的肝硬化患者(诊断为肝癌的假阳性病人)、总 f - A1AT 低的患者和诊断为肝癌的患者四类。见[33]

简而言之,在假阳性(相对于正常的受试者,总的 f - A1AT 增高)的 20 个肝癌病人和 20 个肝硬化病人,核心 F(1,6)A2G2 和外臂 A3F(1G3)岩藻糖结构的 A1AT 通过在散布图中所报道的方法来确定[34]。肝硬化来源的 A1AT 有增高的

外臂,而没有核心结构。在肝癌中,A1AT 核心和外臂都有增高。因此,核心的增高仅在肝癌来源的 A1AT 中出现。

相对于肝硬化但无肝癌的受试者,核心 f－糖形式选择性地在肝癌病人的血液中增高的原因正在研究(数据未显示)。有学者曾设想,核心岩藻糖基化是由在极化的肝细胞中肝糖蛋白被分选到胆汁的机制而形成[35]。我们已假设正因为在肝病中癌细胞已去极化和确定肝细胞极性的紧密连接也被破坏,才导致核心岩藻糖化的糖蛋白被错误分选而且被错误地定向进入血液中。无论是什么原因,我们推测,对癌症特异生物标记的糖基化形式的认知的形成和检测这些特异糖基化形式的能力的发展,例如,A1AT 核心岩藻糖化,将会导致在癌症检测上显著的改进(Block et al,待投稿)。

用病人的人口统计数据和临床信息来解释一个生物标记的水平的意义

我们认为,综合患者的临床情况的分析,再加上一个生物标记的聚糖分析,或者说可能和该生物标记的聚糖情况相关,将会改进一个生物标记的诊断价值。

不幸的是,生物标记的发现和发展通常使用那些缺乏与之相关的人口统计数据和临床因素的样本。而这些因素可能在确定一个生物标记的临床诊断价值上非常相关和有用。例如,在两项研究中,研究人群的临床特点不同,在一个研究,GP73 区分肝硬化和肝癌时表现出很好的差异性,然而在另外一个研究中,GP73 则不然(表 1,研究 4 和 5)。有很多因素在对照组(肝硬化,无肝癌)与患者间(肝硬化,有肝癌)平均临床水平上显示明显的差异。

首先,相对于 GP73 表现差的研究中的病人,生物标记在区别对照和病例表现好的研究中的“对照”(没有检测到肝癌特征)年龄偏小,他们是干扰素治疗有效的患者,而其他的则呈现出较好的代偿性的肝病特征(低水平的 ALT、AST 和 Alk 磷酸酶)。对 GP73 有影响的临床因素总结在表 2 中。

由于上述这些区别可能反映了不同人群不同的临床表现,因此,若是将 GP73 和 f-hem 值与临床因素结合起来考虑,将会提高生物标记在某人群的检测价值。而仅仅单独考虑这些生物标记,则在这些人群的检测价值不大。所以,在研究 4 和 5(表 1)中,从表现差的群体中,年龄和性别相匹配的样本,联合 GP73、f-hem 值,以及临床变量用 Logistic 回归分析来确定:是哪些临床变量显示出肝硬化和肝癌的差异。这些结果表明若是考虑年龄和性别,鉴别因子的价值将会得到显著的改善。例如,在区别肝癌和肝硬化时,将年龄和性别连同 GP73 和 f-hem 的值一起研究,AUROC 的值达到 0.75。因此,尽管这明显低于单独使用 AFP 所达到的 0.81

的值,然而,它却远远优于单独使用 GP73 所得到的 0.65 的值(研究 5,表 1)。

表 2　影响 GP73 水平的临床因素[1]

	Affect upon serum GP73
Increased age	Increases
Increased ALK PHOS[2]	Increases
Increased MELD[3]	Increases
IFN failure[4]	Increases
Liver cirrhosis	Increases

[1] The affects of the patient information shown in the first column upon serum GP73 levels is indicated in the second column. This is based upon our analysis of data from studies 4 and 5 Table 1). The indicated patient variable, alone, was associated with increases in serum GP73 even in the absence of HCC

[2] ALK PHOS is serum alkaline phosphatase, which may be elevated as a result of bile elements in the circulation

[3] MELD, Model for end stage liver disease

[4] IFN, interferon. This is based on HCV patients who have been treated within and failed to reach efficacious clinical milestones, with IFN and ribavirin

将这些因素放在一起考虑,用回归分析可构建出决策树,从而提供在有临床信息背景下对 GP73 水平的解释。图 3 展示了一个用"决策树"来说明 GP73 的水平,同时结合考虑其他临床因素,以分析一个病人的肝癌危险程度。对肝癌而言,利用 GP73 的水平评估危险程度的准确性相当高。

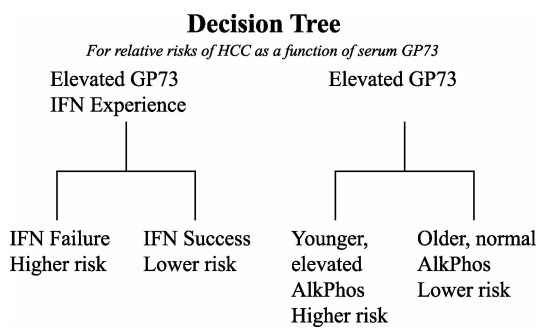

图 3　使用 GP73 值来分析个体肝癌风险率的决策树

个体先由干扰素治疗的作用来分组,然后通过年龄和碱性磷酸酶来进一步区分。特异的几率比值和风险率现在正在计算,将会在别的地方报道(Evan et al 和 Devarajan et al,待投稿)。

结论

虽然先前的一些报告,有些来自于我们团队,表明增高的 GP73 可以区别肝癌和肝硬化,目前的研究却警示我们一个现实问题,就是利用单一的 GP73,在任何一个群体中实现这种区分是不足够的。这是因为很多其他的临床因素可能影响这个生物标记的水平。本研究试图鉴定出其中的一些临床变量,并提供利用这些临床测量值的一种方法,结合 GP73 值,达到最大化地利用整个临床生物标记家族。

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肝癌临床研究的展望

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过去半个世纪肝癌临床研究取得了可喜进步，在病理学基础上，逐步发展了各种消灭肿瘤的疗法，使生存率有了实质性提高。复旦大学肝癌研究所（下称我所）外科过去40余年原发性肝癌住院病人的资料提示，通常每10年都可看到5年生存率的提高（1968—1974为7.4%，1978—1987为25.1%，1988—1997为39.8%，1998—2010为44.0%），说明20世纪肝癌临床与研究成绩肯定。但从整个肝癌人群统计，如美国过去30年，5年生存率1975—1977为4%，1984—1986为6%，1999—2005为14%（Jernal等. CA-Cancer J Clin 2010），说明虽有提高，但离攻克肝癌还有很大距离。展望未来，肝癌临床研究仍任重道远，以下几个方面值得关注。

早诊早治仍然重要

早诊早治仍然是提高肝癌疗效的最主要途径，我所上述每10年生存率的提高，主要归因于早诊早治，因为各阶段肿瘤中位直径分别为11厘米、9厘米、7厘米和5厘米。Altekruse等（Hepatology 2012）报道，1975—1977年的病例5年生存率为3%，1998—2007年提高到18%，也认为主要是因为更多病人得到早诊早治，为此推断如能继续加强高危人群监测，生存率当可能进一步提高。近年已有一些新的肝癌早诊标志物，如高尔基蛋白73（Mao等. Gut 2010）。但荟萃分析认为其准确性与甲胎蛋白相当（Zhou等. BMC-Cancer 2012）。Shang等认为肝癌早诊，骨桥蛋白较甲胎蛋白更敏感（Hepatology 2012）；Tomimaru等也称血浆miRNA－21优于甲胎蛋白（J Hepatol 2012），但用于高危人群的监测，则还有待研究；Giannini等认为甲胎蛋白对于肝癌监测仍有用（Hepatology 2011）。笔者以为，甲胎蛋白合并超声显像对高危人群的定期监测仍然是当前切实可行的办法。我所早期病人比例的增多，主要是年度体检发现的。但由于甲胎蛋白对肝癌只有50%～60%的阳性率，寻找新的、简便易行的早诊标志物仍是重要课题。总之，早诊早治乃事

半功倍之道,是提高肝癌疗效的有长远意义的方向。

综合治疗是长远战略方向,“消灭+改造”的综合模式值得关注,消灭肿瘤疗法成绩肯定,尤其对早期病人

我所小肝癌的5年生存率:切除者为57.9%(共5767例),约为大肝癌切除者的一倍(31.5%,5345例);射频消融治疗为47.9%(482例);符合米兰标准的肝移植为76.9%(193例)。但均未彻底解决问题,需要研究这些“根治性治疗”后残癌的控制。癌症是多基因参与、多阶段形成、涉及多个环节、而且不断变动的复杂疾病,不能期望找到如同治疗细菌性疾病单一的特效药。初步结果已提示,针对单一或少数几个分子的分子靶向治疗的疗效远不如预期。为此,综合治疗是长远战略方向。过去半个世纪的综合治疗模式主要是基于肿瘤是局部病变的“消灭+消灭”模式(如手术+放化疗),这个模式仍有很大的研究空间,如有报道,小肝癌射频消融合并经导管动脉内化疗栓塞(TACE)优于单独射频(Morimoto等. Cancer 2010),等等,但其结果也不如预期。今后改造残癌/微环境/机体疗法的研究将不能或缺,尤其是与消灭肿瘤疗法的综合应用将是一个重要方向,以达到既针对局部,又兼顾整体。

近年已出现多种综合治疗模式,包括合并应用“改造”相关的疗法:(1)合并应用细胞因子切除或消融治疗后合并用α干扰素,可一定程度提高疗效。我所首次发现α干扰素通过抑制血管生成有助预防肝癌转移复发(Wang等. Hepatology 2000),并经临床随机对照研究证实后,其疗效已得到公认。1180例肝癌根治性治疗后的荟萃分析表明,合并干扰素可提高无复发2年生存率35.4%(Shen等. J Hepatol 2010)。也有报道长效干扰素合并动脉内5氟尿嘧啶治疗晚期肝癌3年生存率达44%(Kasai等. Cancer 2011)。(2)合并应用抗炎剂:炎症微环境促癌,已被认为是癌症的第7个特征(Mantovani. Nature 2009)。炎症对癌症的发生、发展、侵袭、转移起决定性作用,它可影响机体的免疫和对治疗的反应(Grivennikov等. Cell 2010);并由此出现或即将出现的一系列可用于癌症的抗炎治疗剂,如阿司匹林和非类固醇抗炎剂、抗细胞因子治疗、阻断激酶的小分子、小核糖核酸等(Dinarello. Cell 2010)。我们的实验也发现用唑来膦酸清除巨噬细胞,通过抑制转移和抗血管生成,可提高多吉美(Sorafenib)治疗肝癌的疗效(Zhang等. Clin Cancer Res 2010)。(3)合并应用分子靶向治疗:多吉美已被认为是治疗晚期肝癌的有效药物,有报道在原位移植模型多吉美抑制肝癌术后复发转移(Feng等. Hepatology 2011)。近年趋势是多吉美与其他分子靶向药物或化疗合用,如多柔比星与多吉美合用优于多柔比星独用(Abou-Alfa等. JAMA 2010)。(4)合并全

身性干预:癌症是全身性疾病,有报道,影响神经、内分泌和免疫的慢性应激可促癌生长和促血管生成(Thaker 等. Nat Med 2006)。为此全身性干预值得重视,全身性干预可通过神经、免疫、内分泌、代谢等诸多途径,目前成熟的研究虽不多,但其发展前景广阔,尤其是在基本消灭肿瘤后合并应用以控制残癌。合并免疫治疗值得特别关注,有报道用天冬氨酸羟化酶(ASPH)负荷的树突状细胞(DC)疫苗,通过 ASPH 刺激产生抗原特异 CD4 + T 细胞,可减少肝癌复发(Shimoda 等. J Hepatol 2012)。肿瘤裂解物刺激的树突状细胞作过继免疫治疗,有些病人观察到甲胎蛋白的下降和 γ 干扰素的析出(Palmer 等. Hepatology 2009)。最新的报道,肝雄激素受体可抑制肝癌转移(Ma 等. Hepatology 2012),甲状腺激素受体可有力抑制癌转移(Liao 等. Hepatology 2012)等,均提示内分泌干预的可能。长效精氨酸脱亚氨酸可使晚期肝癌病情稳定(Yang 等. Br J Cancer 2010)等,也为代谢干预提供线索。(5) 合并中医中药:中医中药消灭肿瘤的作用可能较弱,而"改造"的作用可能有其地位,从而达到使癌细胞改邪归正,带瘤生存。常用于肝癌的中药复方,实际上可能包括抗炎、改善微循环、抗血管生成、提高免疫等的综合作用。中医辨证论治也可能有助探索整体水平的个体化治疗。我们实验研究发现含 5 味中药的"松友饮"可下调基质金属蛋白酶 2(MMP2)和血管内皮生长因子(VEGF),诱导凋亡,对肿瘤生长有一定抑制作用并延长荷瘤鼠生存期;还可上调表皮 - 钙黏蛋白(E-cadherin)、抑制由奥沙利铂化疗诱导的转移并延长生存期(Xiong 等. BMC-Cancer 2010)。(6) 合并潜在的治疗:近年出现了一系列新的治疗靶点和线索,如小 RNA、肝癌相关分子(尤其是信号通路和肝癌干细胞相关分子)、免疫和内分泌的分子调控、内皮细胞正常化、抗有丝分裂途径,以及应用一些"无关药物"。如治疗结核病的利福平可作为肝癌抑制血管生成的口服药物(Shichiri 等. Cancer Res 2009)。这些潜在的治疗中,小 RNA 值得关注,如 miR - 139 通过下调 Rho-kinase2 抑制肝癌转移(Wong 等. Gastroenterology 2011),miR - 7 干扰 P13K/AKT 通路抑制肝癌转移(Fang 等. Hepatology 2012),miR - 135 则促进肝癌转移(Liu 等. J Hepatol 2012)等,均有潜在治疗作用。

消灭肿瘤疗法促残癌转移

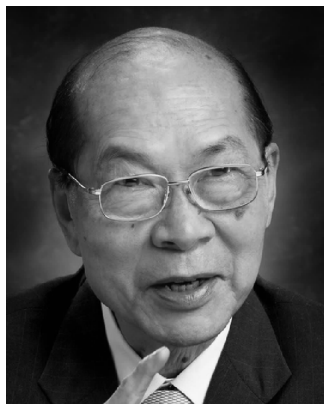
我们实验提示常用于肝癌的消灭肿瘤疗法均有促残癌转移的负面问题:姑息性肝癌切除可上调 MMP2/VEGF 而促进残癌转移;放疗上调 TMPRSS4 促进远期转移(Li 等. Cancer Gene Ther 2011);奥沙利铂化疗下调表皮 - 钙黏蛋白而促进转移;肝动脉结扎因乏氧激活 β-catenin,诱发上皮 - 间质转化而促进转移(Liu 等. Clin Cancer Res 2010);多吉美也同样促进残癌的侵袭播散。近期文献已有不

少类似报道:阻断肿瘤血供有时可加速肿瘤播散(Hayden. Nature 2009);抗血管生成剂抑制肿瘤生长,但促进转移(Loges 等. Cancer Cell 2009)。为此,对目前常用的消灭肿瘤疗法既要肯定其疗效,也要关注其负面问题而研究对策,这是提高现有疗法疗效的一条捷径。我们的实验研究初步发现,合并应用一些常用药物可减轻这种负面作用,如干扰素、中药小复方"松友饮"、酪丝亮肽、唑来膦酸、阿司匹林、苦参素、丹参酮等。

肝癌转移复发是瓶颈

文献和我所资料均提示,肝癌各种疗法的 5 年生存率(肝移植 60% ~80%,小肝癌切除 50% ~60%,大肝癌切除 30% ~40%,小肝癌消融疗法 30% ~40%,经导管动脉内化疗栓塞 20% ~30%)都已接近其高限,瓶颈主要是转移复发。我所小肝癌切除 40 年来疗效没有提高,每 10 年的 5 年生存率依次为 57.9%、57.9%、55.5% 和 58.1%,问题是对转移复发没有新办法。所幸者,近年在癌转移方面,出现了一些新的认识:(1) 肝癌转移不是晚期现象:研究提示,肝癌转移潜能起源于原发瘤,即使小肝癌也可有很高的转移潜能(Ye 等. Nat Med 2003)。(2) 肝癌转移与免疫炎症微环境关系密切:发现癌周肝 17 个免疫炎症相关基因可预测转移(Budhu 等. Cancer Cell 2006)。为此,癌转移研究不能只针对癌细胞,如何改造"土壤(微环境)"将大有可为。(3) 肝癌转移潜能可以双向变:即可变坏也可变好。如上述消灭肿瘤疗法可促癌转移;反之,也看到一些治疗可降低转移潜能。(4) 肿瘤干细胞在癌转移中起重要作用。干细胞与微环境基质细胞相互作用才能形成转移灶(Malanchi 等. Nature 2012)。(5) 转移过程癌细胞基因呈动态变化(Clifford. Nature 2012)。预期所有这些将为设计抗转移疗法提供线索。

总之,肝癌临床研究仍任重道远,消灭肿瘤仍然是基本的,而改造肿瘤和机体则是重要的补充,预期生物学将是进一步提高肝癌生存期的关键因素。



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国际著名肝癌研究学者,肝癌早诊早治奠基人。中国工程院医药卫生工程学部首批院士,美国和日本外科学会名誉会员。现任复旦大学肝癌研究所所长、复旦大学附属中山医院肿瘤外科教授、博士生导师,曾任国际抗癌联盟理事、中国抗癌协会肝癌专业委员会主委、中华医学会副会长、上海医科大学校长。

汤院士在国际上最早系统性地提出“亚临床肝癌”概念,主编英文版《亚临床肝癌》专著,国际肝病学奠基人 Hans Popper 称“这一概念是人类认识和治疗肝癌的重大进展”。它使肝癌手术切除后 5 年生存率提高一倍,使肝癌从“不治之症”向“部分可治之症”转化。近年来又投入“肝癌转移复发的研究”,在国际上最早建成转移性人肝癌裸鼠和细胞模型,并成功用于肝癌转移的研究。2 次任国际癌症大会肝癌会议主席,90 余次在国际会议作特邀演讲,主办 7 次上海国际肝癌肝炎会议并任主席。任 11 本国际杂志编委,2 本亚太区杂志主编。主编专著 9 本,参编国际专著 16 本。发表 SCI 肝癌研究论文 288 篇,被引用 6759 次,在肝癌领域全球排名第 3(大陆第 1)。1979 年获美国癌症研究所“早治早愈”金牌,由此奠定了我国在肝癌研究领域的国际地位。以第一作者获国家科技进步一等奖 2 项、三等奖 2 项,何梁何利科技进步奖,中国医学科学奖,中国工程科技奖,吴阶平医学奖。还曾获白求恩奖章、全国“五一”劳动奖章、上海市科技功臣等荣誉称号。培养并已毕业博士生 59 人,4 人获全国优秀博士论文奖。

非可控性炎症与代谢异常促进肝癌发生

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肝癌是世界第五大常见肿瘤,其死亡率在恶性肿瘤中居第三位。由于缺乏特异的根治性治疗手段及早期预测和诊断方法,肝癌患者的预后较差。目前肝癌发生发展与复发转移的机制尚未阐明。

肝炎是肝细胞癌最常见的病因,占肝细胞癌发病率的75%。在长期的癌前病变过程中,肝脏经历肝纤维化、肝硬化,逐渐产生分化异常的肝细胞,最终引起肝细胞癌的发生。尽管肝细胞癌的恶性表型在遗传学和/或表观遗传学上表现出一定的异质性,但肝脏的慢性炎症与肝细胞的恶性转化相关已十分明确。大量研究表明,肝脏非可控性的炎症与肝细胞癌的发生紧密相关,且已知炎症微环境在肝细胞癌的发生发展中发挥重要的作用。炎性细胞的浸润可能由肝癌细胞或坏死/凋亡的肝细胞引起。免疫细胞分泌的各种细胞因子、趋化因子以及免疫细胞与肝癌细胞表面蛋白间的直接接触重塑了肝脏的微环境,促使肿瘤细胞发生基因突变及增殖。肿瘤坏死因子与白介素是肝癌微环境中研究最多的信号分子。这些细胞因子能够激活多种转录因子,如 NF-κB、STAT3、AP-1 等。通过调节多种下游靶基因的表达,调控肿瘤发生、转移与血管生成。只有掌握预测和阻遏非可控性炎症的新策略,才有可能使肝癌的发病率下降。

研究表明,肿瘤来源于一小群称为肿瘤起始细胞或肿瘤干细胞的细胞。这些细胞是肿瘤中一个特殊的亚群,具有自我更新能力、分化能力和体内成瘤性等至少三种特有性能,参与肿瘤的起始与进展过程。大量有关肝癌异质性的证据提示,肝癌中也存在这类肿瘤干细胞,可促进肝癌的生长和转移。成人的肝细胞具有干细胞样的特征,因为它们在肝脏受损时具有广泛增殖的能力,并且在特定条件下能够分化为胆系细胞。因此,肝细胞在适当的微环境中可能会通过去分化而转化为肝癌干细胞,启动肝细胞癌的发生。肝癌干细胞的另一种可能的细胞来源是调控异常的肝干/前体细胞。我们最近的研究证明了 HBx 能促进肝前体细胞

的扩增并导致肝癌干细胞的产生;而如 p28 这样的癌蛋白可经十分复杂的信号途径促进肝癌干细胞的干性扩增。

一些代谢因素,如糖尿病、肥胖、酗酒与摄入有毒食物等,与包括肝纤维化和肝细胞癌在内的慢性肝病的关系已成为近年的研究热点。代谢综合征(MS)是目前世界上最严重的公共卫生问题之一,受到广泛关注。MS 泛指机体发生代谢异常的各种表型,包括腹型肥胖、高脂血症、糖尿病及高血压。同时 MS 的发生还伴随着胰岛素抵抗综合征,该疾病与慢性炎症的状态密切相关。此外,肥胖和糖尿病还被证明是 HCC 明确的危险因子。最近一篇综述系统分析了 13 个病例对照研究,其中 11 个病例研究证实了糖尿病和 HCC 发生之间的关联。在这些病例中,患糖尿病的研究对象较对照组而言,其肝癌的发生概率显著升高了 2 倍。引起肥胖症的主要信号通路也与非酒精性脂肪肝病(NAFLD)相关。NAFLD 是西方国家引起成人慢性肝病的最常见原因之一。NAFLD 包含一系列症状谱,从脂肪沉积、炎症发生、气球样变性、到非酒精性肝炎(NASH),后者是公认的肝硬化及肝癌发生的原因之一。

与肝实质的代谢综合征相似,NAFLD/NASH 是 HCC 的高危因素,这一影响与肝硬化无关。一项研究表明 NAFLD 是 HCC 最主要的危险因素。越来越多的证据也表明内脏脂肪组织分泌血管内皮生长因子(VEGF),表明血管异常生成可能导致肥胖患者较差的临床预后。在动物模型中,瘦素活化了 HCC 细胞的许多信号通路,例如 JNK、PKB、AKT 及胞外信号调节激酶通路,这些通路均可促进肿瘤的进展。酗酒也可通过诱发和加重肝硬化的水平增加 HCC 的发生风险,而摄入咖啡似乎可以预防包括 HCC 在内的肝脏疾病。目前已有一些假说可以解释这一现象。随着生活水平的提高和生活方式的改变,我国肥胖人群、脂肪肝和糖尿病患者大量增加,增加了患严重肝病、肝癌的风险,需要及早防范。

肝癌是一种由环境因素和遗传因素共同作用的复杂疾病。既然肝炎是肝癌的重要病因,那么抗乙肝病毒的治疗对肝癌的预防非常重要。遗传和组织的异质性使目前肝癌的治疗很不理想。因此,肝癌的分子分型有望为将来的个体化治疗提供依据。对各种不同的因素进行整合和综合分析对改进肝癌的治疗非常重要。小分子靶向药物与常规化疗的联合应用可以提高肝癌的治疗效果。针对肝癌不同靶点的多靶点药物联用可取得最佳的治疗效果。尽管目前转化医学的兴起极大地促进了肝癌基础研究的成果向临床应用转化,总体而言,肝癌诊疗的研究依然面临挑战,任重而道远。

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肿瘤学、分子生物学专家。教育部“长江学者奖励计划”特聘教授，中国工程院院士、发展中国家科学院院士。德国乌尔姆大学博士，德国 Max-Planck 研究所生化所博士后和 PI。现任第二军医大学肝病研究中心主任、生物信号转导研究中心主任，上海东方肝胆外科研究所常务副所长。兼任国家自然科学基金委员会医学科学部主任、中国生化学会副理事长。

长期从事恶性肿瘤的基础与临床研究，对肿瘤的信号网络调控、肝癌新分子标志物鉴定及应用等有重要建树。在 *Cancer Cell*、*J. E. M.*、*Gastroenterology*、*Hepatology* 等发表论文百余篇；获国内外发明专利授权 10 项。获国家自然科学二等奖、何梁何利科技进步奖等。

慢性乙型肝炎病人的新型个体化治疗

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慢性乙型肝炎病毒感染在全世界范围都是一个重大的健康难题,中国尤甚。据估计,每年有 50 万中国人死于终末期乙型肝炎并发症,造成了巨额的医疗费用和沉重的社会经济负担。慢性乙型肝炎治疗的目标是通过预防疾病向肝硬化、终末期肝病、肝细胞肝癌及死亡进展来提高生活质量和延长生存期。有两类公认的抗乙肝病毒的药物,分别是干扰素(包括普通干扰素和两种聚乙二醇干扰素)和核苷(酸)类似物(拉米夫定、阿德福韦酯、恩替卡韦、替比夫定和替诺福韦)。

依靠发现有效的应答预测指标,我们应该做的主要工作之一是提高抗病毒疗效和减少由于对现有抗病毒策略应答不良而造成的病毒耐药。优化抗病毒策略的关键是在合适的时间点选用合理的药物开始治疗、密切监测治疗应答反应和对于治疗应答不佳的病人,考虑加入第二种药或更换另一种更加有效的药物。

慢性乙型肝炎病人的优化抗病毒策略包括基于应答指导(RGT)的干扰素治疗或基于核苷类似物的治疗[选用高效价、低耐药的核苷类似物开始治疗,或选用高耐药率(如拉米夫定、替比夫定)或低效价抗病毒作用(阿德福韦)的核苷类似物开始治疗,然后根据病人应答情况调整治疗策略(路线图概念)]。

基于应答指导(RCT)的干扰素治疗

在中国进行了一项旨在明确如何应用 RGT 优化聚乙二醇干扰素 α-2a 治疗 e 抗原阳性患者的方法。初步结果显示,261 名患者完成了 24 周的聚乙二醇干扰素 α-2a 的治疗,其中 25%(66/261)的患者达到了早期应答的标准(聚乙二醇干扰素治疗 24 周后 HBV DNA < 5 Log copies/mL,并且 HBsAg < 1500 IU/mL)。在取得早期应答的患者中,26%(17/66)的患者在治疗 24 周时发生了 e 抗原血清学转换,而非早期应答者发生 e 抗原血清学转换的比例只有 6%。早期应答者的基线表面抗原/e 抗原定量和 HBV DNA 明显低于非早期应答者。早期应答者的表

面抗原、HBV DNA 和 e 抗原与基线相比的下降水平(分别是 -1.48 log IU/mL, -4.78 log copies/mL, -1.57 PEIU)比在非早期应答者(分别为 -0.41 log IU/mL, -2.47 log copies/mL, -0.82 PEIU)更加明显。这项研究将对慢乙肝病人如何从基线指标和应答指导的治疗中获益提供有价值的信息,来帮助优化治疗持续时间。这项研究最终的结果令人期待。

初始治疗患者的核苷类似物治疗

恩替卡韦和替诺福韦具有高效价、低耐药的特点,已经被主要的治疗指南推荐为一线药物(AASLD 2010 和 EASL 2012),或者作为初始治疗患者的优先选择(中国指南 2010 和 APASL 2012。但是由于一线药物高昂的价格,只有 13% 初始治疗患者把恩替卡韦作为首选的抗病毒治疗药物,而超过 70% 的患者仍在选用拉米夫定、阿德福韦或替比夫定治疗,导致了大批病人出现应答不佳和/或病毒耐药。

基于核苷(酸)类似物的路线图/救援策略

如果患者把高耐药的药物,如拉米夫定和替比夫定作为起始治疗药物,应该给予密切的监测。如果出现耐药,应根据病人的应答情况调整治疗方案,加入另一种无交叉耐药的核苷类药物。根据最优治疗策略,大约 2/3 的患者需要加用其他核苷(酸)类似物。同时,足够的实验室支持和良好的病人依从性也是治疗成功的关键。目前,一个关键的随机对照研究(EFFORT study)正在中国进行,接受优化治疗方案的患者与那些接受标准治疗的患者相比,更多的患者达到 HBV DNA < 300 copies/mL[71.7%(215/300)和 58.5%(175/299),$P = 0.001$],病毒学突破更低[1.7%(5/300)和 12.0%(36/299),$P < 0.001$],基因型耐药更低[1.3%(4/300)和 10.7%(32/299),$P < 0.001$]。根据治疗 24 周时的应答情况而采用加药治疗(add-on)的路线图观念被证明是有效的,因为和标准治疗方案相比,采用这种加药治疗的患者在治疗 76 周时获得了更加明显的抗病毒效果,减少了病毒学突破率。

未来的展望(EASL 和 APASL 指南)

在未来,基于国家科技重大肝炎研究专项课题的启动,更多的关于优化抗病毒药物疗效的重要数据将从中国产生。随着经济的发展和医疗系统的改革,包括新的医疗补偿政策的实行,将使得中国的慢性乙肝患者更容易接受抗病毒药物治疗,最终也会使更多中国医生可遵照国际和中国慢性乙肝治疗指南或推荐意见而

治疗慢性乙肝患者。

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侯教授目前为中华医学会感染病学分会候任主任、南方医科大学附属南方医院感染内科主任。侯教授的主要研究方向为病毒性肝炎的临床管理和乙肝病毒感染的分子病毒学和免疫学。先后在《Hepatology》,《Science》,《Journal of Hepatology》等杂志上发表论文200余篇。

展望慢性乙型肝炎的新治疗方法

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世界卫生组织估计全球有20亿人感染过乙型肝炎病毒(HBV)。自从预防性乙型肝炎疫苗免疫在超过170个国家应用以来,乙型肝炎病毒新感染率已持续下降。虽然,预防性疫苗取得了巨大成功,但慢性乙型肝炎却始终是一个全球性健康问题。全球有3.6亿人处于HBV的持续感染状态,其中每年有100万人死于HBV相关的肝硬化及肝癌。由于政治与经济的不稳定,有些国家乙型肝炎疫苗的免疫效果不尽如人意。乙型肝炎病毒感染的结局因人而异,个体差异较大。成年期感染,大多可以自然清除,仅约5% ~10%发展成慢性乙型肝炎。相反,40% ~90%由母婴传播的感染终会发展为慢性肝病[1].

近年来,慢性乙肝的治疗取得了显著进展,两类药物被批准用于临床,包括长效干扰素(PEG－IFNα)和核苷类似物(阿德福韦、恩替卡韦、拉米夫定、替比夫定和替诺福韦)[2-5]。然而,上述药物在预防肝硬化和肝癌上效果甚微。即使与核苷类似物联合应用,长效干扰素也仅能在约三分之一的患者中诱导持续的抗病毒效果。另一方面,核苷类似物可以显著地抑制病毒复制,并缓解肝脏的炎症,但不能彻底清除病毒。绝大部分患者在停药后会出现病毒反弹。长期服药可以导致耐药,并最终导致治疗失败[6,7]。因此,新的慢性乙肝治疗途径亟待研发。

适度的适应性免疫被证明是有效控制乙型肝炎所必需的。解决感染的关键是T细胞介导的、针对病毒的直接免疫应答[8-12]。乙型肝炎病毒特异性的$CD8^+$ T细胞可以通过分泌Th1型抗病毒的细胞因子,诸如IFNs、TNFα、Fas－Fas－L,来清除乙肝病毒感染的肝细胞[12-16](图1)。针对病毒蛋白的早期、有力、多克隆的特异性细胞免疫反应是在感染的早期清除乙肝病毒所必需的。相反,在病毒持续感染的携带者体内表现的是弱而一过性的、甚至是无法检测到的$CD8^+$ T细胞应答[17-21]。体液免疫,特别是能中和病毒包膜蛋白的抗体在防止乙肝病毒扩散到未感染的肝细胞方面发挥重要作用[20,22]。

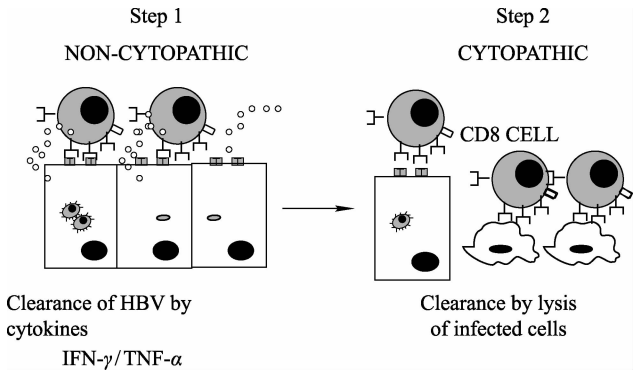

图 1　通过 CTL 控制 HBV 感染，通过细胞因子（IFN－α 和 IFN－γ）抑制 HBV 复制，通过 HBV 特异性 CD8 T 细胞的细胞毒活性清除感染细胞［Desigse，A. Bertoletti］

最近的研究表明，慢性乙肝患者特异性 T 细胞免疫失能的过程中，有一系列机制。高浓度的病毒载量对病毒特异性免疫具有负面影响。肝脏中病毒的高复制，导致病毒载量高于 10^7 copies/mL，与慢性乙肝患者病毒特异性 $CD8^+$ T 细胞反应相关[23]。甚至，在慢性感染过程中，长期暴露于病毒抗原将导致 T 细胞免疫耐受，而易于凋亡（图 2）。程序性死亡受体 1（PD－1）与其配体 PD－L1（亦即 B7－H1）的相互作用在防止免疫系统过度反应中发挥重要作用[24]。近期研究还揭示了抑制性分子如 PD－1 和 CTLA－4 可以在病毒特异性 T 细胞明显上调，导致这些细胞衰竭（如生产 IFNγ，不增殖）[25]。同时，该机制还能通过损伤有效的抗病毒反应促进慢性感染的发展。这一假设曾在丙型肝炎病毒[26-27]、HIV[28-30]以及小鼠 LCMV 感染[31,32]中得以证实，最近在 HBV 感染中也被证实[33,34]。进一步研究还发现，抗原递呈细胞，特别是树突状细胞的失能，促成了慢性乙型肝炎患者的 T 细胞免疫损伤[35-41]。体外实验发现，较之健康对照，自慢性乙肝患者体内分离的 DC 细胞只能产生较低水平的抗病毒细胞因子，如 α 型干扰素与肿瘤坏死因子 α[35,36]。这种 DC 细胞无法有效活化 T 细胞、刺激 T 细胞增殖[35,39-41]。更新的研究还证明，来自慢性乙肝患者骨髓的 DC 细胞表达高水平的抑制性 PD－L1 分子，导致乙肝病毒特异性 T 细胞功能下调[39]。

一些研究强调了 $CD4^+$ $CD25^+$ 调节性 T 细胞在持续性感染发病机制中的重要性[42]。在 HCV 及 HIV 感染的患者体内，调节性 T 细胞可以下调 HCV 与 HIV 特异性的 $CD8^+$ 细胞，从而影响疾病的进程[43-45]。调节性 T 细胞在慢性乙肝感染中的作用尚不清楚。然而，在重症慢性乙肝患者的肝脏与血液中检测到 $CD4^+$ $CD25^+$ 调节性 T 细胞异常升高[46]。小鼠实验证明调节性 T 细胞通过限制细胞因子的分泌，下调效应性 T 细胞的抗病毒效果[47]。此外，肝脏作为一种具有耐受原

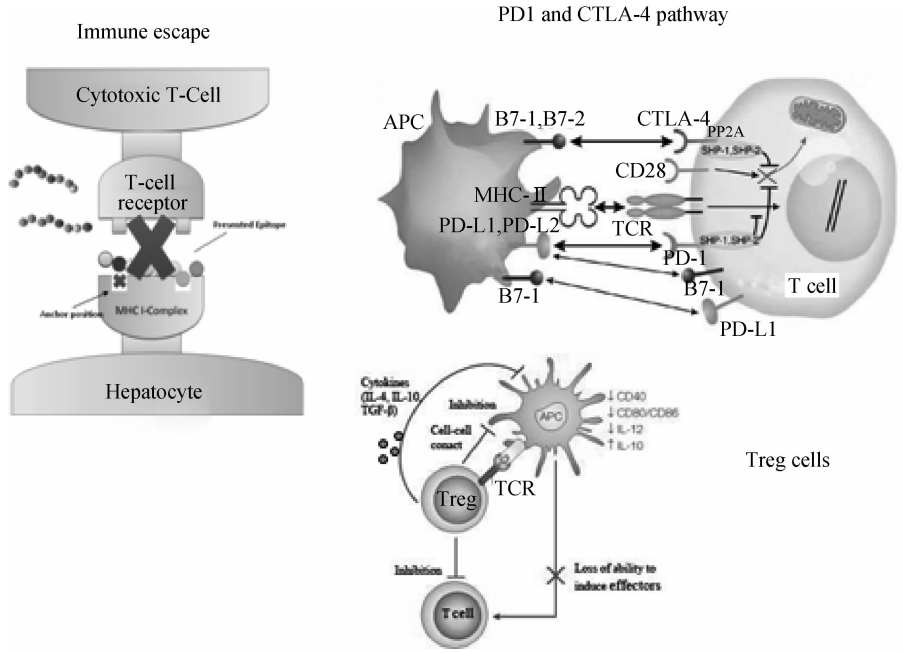

图 2　免疫逃避的机制:CD8⁺ T 细胞失能(Nature Immunol 3,2201－2205)

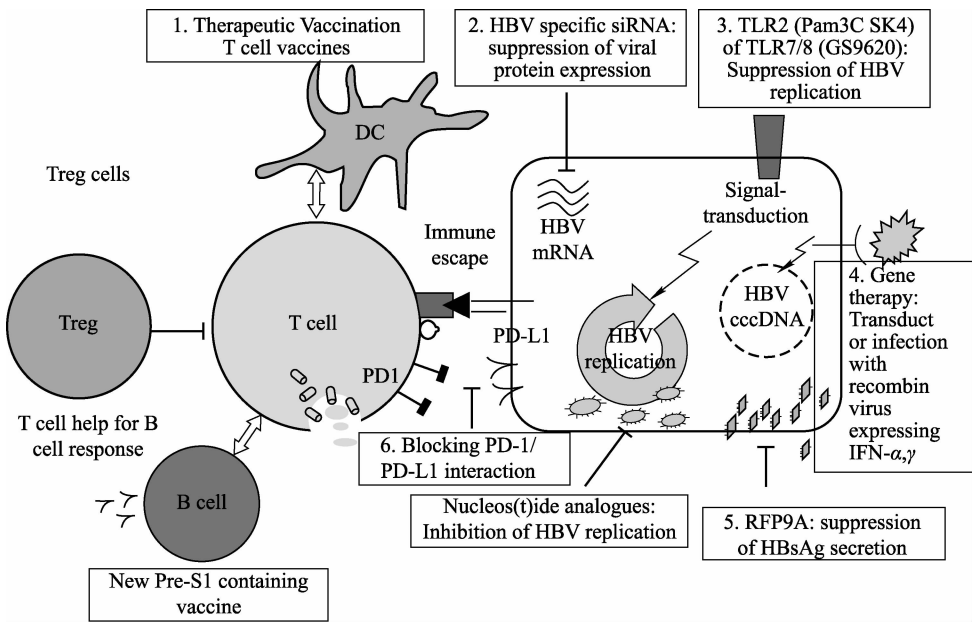

图 3　联合应用乙肝治疗新途径可能是消灭 HBV 的必由之路

性质的器官,可能也参与了慢性乙肝免疫耐受的形成[48,49]。最后,病毒自身发展出来的有效逃避宿主免疫反应的策略也导致了持续性感染。在 HIV、HCV 和 HBV 感染者中均观察到因 HLA 相关的 CD4⁺、CD8⁺ T 细胞以及 B 细胞表位变异导致病毒免疫逃避[50－55]。

研究表明单独使用拉米夫定,或联合使用白介素 12(IL－12)可以促使 HBV 特异性的 $CD4^+$ 和 $CD8^+$ T 细胞免疫反应恢复,但疗效不能持续[56－58]。

以下,我们将讨论可能应用于治疗慢性乙肝的新治疗策略,包括:

1. 新的免疫治疗方法
2. HBV 特异性小分子干扰 RNA(siRNA)
3. Toll 样受体刺激固有免疫反应
4. 基因治疗方法
5. 新的抗病毒分子抑制表面抗原的分泌
6. 免疫调节方法

一、慢性乙肝的免疫治疗

在过去的 20 年里,科学界致力于发展慢性乙肝治疗性疫苗,以增强对病毒的特异性免疫应答,从而治愈 HBV 的持续性感染[59－72]。

众多的临床试验发现,利用现有基于乙肝表面抗原的预防性蛋白疫苗治疗乙肝,可以在部分患者中诱导 HBV 特异性的 T 细胞反应,降低病毒载量,导致 E 抗原血清学转换,然而,效果均表现为一过性,不能有效控制乙肝病毒慢性感染[59－66]。在此基础上联合拉米夫定等抗病毒药物治疗亦无法提高疗效[67－69]。

一些针对刺激 HBV 特异性 T 细胞反应而设计的治疗方法也未获成功[70－72]。包含单个源自 HBV 核衣壳杀伤性 T 细胞表位的多肽疫苗可以在正常人中诱导有力的、初级 HBV 特异性 T 细胞反应[73],但在慢性乙肝患者中却只能产生微弱的 CTL 活性,以至于不能有效地降低病毒载量,诱导 E 抗原的血清学转换[70]。DNA 疫苗可以表达小的及中等的包膜蛋白,诱导 HBV 特异性细胞免疫反应,但仅为一过性[71]。

Yang 等报道了采用新型 DNA 疫苗联合拉米夫定治疗慢性乙肝[72]。这种疫苗包含 5 个质粒,编码大多数的乙肝病毒抗原和人 IL－12 基因作为分子佐剂。联合治疗在 12 个慢性乙肝患者中的 6 个产生了持续的抗病毒反应。在 52 周随访结束时,反应者表现为 E 抗原的清除和病毒载量下降至不可检测,以及检测到至少针对一个病毒抗原的 T 细胞反应[71]。当然,该疫苗的疗效需要在大规模慢性乙肝患者中进一步验证。

由 WEN 等研发的抗原抗体复合物型治疗性乙肝疫苗可使 Fc 受体增强抗原递呈细胞对表面抗原的摄取,调节抗原递呈细胞对表面抗原的处理与递呈,从而诱生有力的 T 细胞反应[74]。临床研究表明该疫苗用于 E 抗原阳性的慢性乙肝患者,可以导致血清中病毒载量下降,E 抗原血清学转换[75]。目前,这是唯一在慢

性乙肝患者中进入了三期临床的治疗性疫苗[76]。即便如此,该疫苗在 IIIA 临床试验中仅在约 15% 的患者中产生了 E 抗原血清学转换,预示着 HBV 的清除。后续的研究将进一步确证该疫苗的疗效。

最近,在法国进行了一项旨在用核苷类似物与 DNA 疫苗联合,诱导针对乙肝表面抗原的特异性 CD4/CD8 T 细胞反应的研究。在该双盲试验中,32 个经核苷类似物治疗病毒载量转阴的患者接受了 5 次 DNA 疫苗免疫,结果停止治疗后,试验组复发率与单用抗病毒治疗的对照组没有差别[Pol S. ,Michel M. ,私人交流]。因为核苷类似物可以减少病毒复制,但无法打破免疫耐受,从而清除病毒。针对核心蛋白或聚合酶蛋白的 T 细胞免疫可能更为有效。

(一) 研究治疗性乙肝疫苗的动物模型

一直以来,人们建立了不同的动物模型用于研发、评价新型治疗性疫苗,包括黑猩猩、土拨鼠、鸭子以及转基因鼠。用编码不同 HBV 蛋白的表达质粒构成的 DNA 疫苗免疫转基因鼠可以诱导 HBV 特异性抗体,并刺激 CTL 反应。然而,HBV 特异性 CTL 的功能在转基因鼠上不能完全表现[77-79]。改变 DNA 疫苗的免疫程序[80]并阻断 PD-1/PD-L1 的干扰[34,81](见下)可以在体内增强功能性 T 细胞反应,抑制病毒复制,但不导致肝炎。因此,能发生嗜肝 DNA 病毒自然感染的动物模型是研究疫苗长期疗效所要求的。相比较黑猩猩而言,土拨鼠模型较为容易获得,并且经济。

用表达土拨鼠乙肝核心抗原(WHcAg)的 DNA 疫苗联合抗病毒药物治疗土拨鼠可以长期控制病毒的复制[82,83]。然而,非常清楚,克服 WHV 持续性感染需要更强有力的治疗性疫苗。一种新型以重组腺病毒为载体的 DNA 质粒(pCGWHc)表现出 WHcAg 的高表达。与先前的 DNA 疫苗[Kosinska A. et al. ,in review]相比,该新型疫苗采用初免-加强的程序可以在小鼠上诱生更强的 WHcAg 特异性 $CD8^+$ T 细胞反应。而且,该疫苗免疫易感土拨鼠,可以诱生显著的 CTL 反应,并诱生抗 WHs 抗体,在用 WHV 攻毒后,清除 WHV 感染。当该疫苗联合核苷类似物治疗 WHV 慢性感染的土拨鼠[Kosinska A. et al, unpublished results],试验组所有土拨鼠能观察到明显的 WHcAg 和 WHsAg 特异性 T 细胞反应。甚至,试验组 4 只土拨鼠中的 2 只在治疗结束到随访结束期间,一直保持 WHV DNA 阴性及抗 WHs 抗体阳性。这些充满希望的数据说明,先用抗病毒药物降低病毒载量,然后采用有效的针对 HBV 核心蛋白的 T 细胞疫苗可能是将来治疗慢性乙肝的方法。

二、HBV 特异性小分子干扰 RNA(siRNA)

采用长度为 21 ~23 核苷酸的小分子干扰 RNA 进行 RNA 干扰亦可能是特异性治疗 HBV 感染的有效方法[84]。在化学合成或载体表达的 siRNAs 介导下,目标信使 RNAs(mRNAs)可以被特异性降解。许多临床相关病毒,如 HIV、HBV、HCV 均能在体外实验中得以抑制[85-87]。某些 siRNAs 已被证明能在暂时或稳定转染系统,抑制 HBV 基因的表达与复制。我们也曾证明两个针对 WHV 基因 S 与 X 区的 siRNAs 能减少土拨鼠原代肝细胞中 70% 的 WHV 转录与复制中间体[88]。此外,在 siRNA 介导 WHV 抑制后,参与固有免疫与特异性免疫的细胞基因表达上调,如 MxA 以及 I 类 MHC。由此,通过对 HBV 复制的抑制,及抗病毒相关的细胞基因表达增强,siRNAs 可能被用于慢性乙肝的治疗。当然,siRNA 在体内如何有效地投递,尚待进一步优化,以取得满意疗效。

三、Toll 样受体刺激固有免疫反应

固有免疫是人体首次遭遇病原体,制止病毒感染的第一道防线。能在感染细胞中建立抗病毒状态,通过产生细胞因子和炎症趋化因子激活免疫细胞,从而限制病毒复制并协调适应性免疫。哺乳类动物细胞配备有大量种系的病原体识别受体,特别是 Toll 样受体(TLRs)可以识别一系列进化上的、高度保守的结构,所谓的病原体相关的分子模式。基于 TLR 配体刺激免疫细胞与体细胞的能力,通过体内或体外检测 TLR 配体,可以判断候选药物直接抗病毒和间接的免疫调节作用。在多个系统中 TLR 配体表现了抗 HBV 的活性[89]。这种活性包括诱导出 IFN 依赖与非依赖性的抗病毒机制。最近又发现了 Toll 样受体 2(TLR2)在慢性乙肝发病机制中扮演了重要角色[90,91]。在患者和土拨鼠模型的外周血细胞中(PBMCs),以及 HBV 慢性携带者肝组织中均发现 TLR2 表达较低。在土拨鼠模型,PBMCs 中 TLR2 的表达与急性 WHV 感染以及用恩替卡韦治疗的慢性 WHV 携带者的 WHV DNA 滴度负相关。TLR2 配体可以活化 NF-κB、PI3K/Akt,以及 MAPK 不同的信号通路,诱导肝细胞产生促炎细胞因子。TLR2 介导的固有免疫反应可以导致人肝细胞瘤细胞以及土拨鼠原代细胞中 HBV/WHV 复制与基因表达的下调。这些研究表明,HBV 复制和 TLR2 信号通路的相互抑制是 HBV 感染的重要机制。

此外,许多 TLR 配体同时也是理想的疫苗佐剂,被用于预防性与治疗性 HBV 疫苗的组分。

四、基因治疗

IFN－α 和 IFN－γ 在急性乙肝痊愈过程中扮演着重要角色。它们能抑制嗜肝病毒的复制。肝内 IFN 水平的高表达可以强化抗病毒活性。这种抗病毒效果曾在慢性乙肝患者中得到证实。然而，患者中抑制病毒的复制率、e 抗原消失率、s 抗原消失率仅分别为 37%、33% 与 8%[92]。此外，由于严重的副作用，大量慢性乙肝患者不适用 IFN－α 治疗，如晚期肝硬化患者[93]。这些副作用包括流感样症状、体重下降、骨髓抑制、发生败血症的风险（特别是肝硬化患者）、脱发、甲状腺功能不全、抑郁症及其他精神性疾病等[94]。使 IFNs 在局部表达，并发挥作用，可能是减少副作用、增强抗病毒效果的新治疗途径。高容量或依赖辅助腺病毒（HD－Ad）载体似乎是投递细胞因子基因到肝脏的好选择。依赖辅助腺病毒载体由于不表达任何病毒蛋白而具有更好的安全性；并且载体诱导的自身免疫反应较之上一代的腺病毒载体有了显著下降[95]。采用依赖辅助腺病毒载体投递基因可以诱导持续的蛋白表达。wIFN－α 和 wIFN－γ 在土拨鼠慢性嗜肝病毒感染中的效果，经采用新途径进行了测试：通过依赖辅助腺病毒载体投递各个细胞因子基因到肝细胞，我们期望在体内肝脏中 IFN－α 和 IFN－γ 有局部持续表达。在 WHV 携带者肝脏体内转染 HD－AdwIFN－α 或者 HD－AdwIFN－γ，可以诱生在动物血清中检测到的、具有生物活性水平的 IFN。wIFN－α 在肝脏的表达降低了肝内 WHV 的复制，使得全部 2 只土拨鼠血清病毒载量保持在 1 个 log 的水平[96]。其它的载体或亲肝重组病毒，如甲肝病毒或丁肝病毒，可能也可用于投递 IFN－α 或 IFN－γ 的基因，但目前尚未有关于重组/减毒的甲肝病毒或丁肝病毒做载体的报道。

五、新的抗病毒分子抑制感染肝细胞乙肝表面抗原的分泌

感染的肝细胞可以释放大量的完整病毒 dane 颗粒及 22 nm 仅包含包膜蛋白的小颗粒。核苷类似物治疗可以抑制病毒复制，却不能抑制 22 nm 小颗粒的释放。正是这些与 HBV 特异性免疫缺陷相关，造成 HBV 感染在肝脏的持续存在的 HBsAg 颗粒导致了免疫耐受。抑制 HBsAg 颗粒的释放有可能使人体重获 T 细胞及 B 细胞反应功能。近期，有关一种新的化合物抑制 HBsAg 在鸭乙肝［Gilbert A.，unpublished results］及乙肝患者（press release REPLICOR）体内释放的初步结果被报道。

REP 9AC’代表了最新的一类可以阻断表面抗原释放的抗病毒临床候选药物。在临床前鸭乙肝模型的研究中，该候选药物表现了显著的抑制作用。同样在

慢性乙肝患者也能很快清除血清中的表面抗原。首个接受试验患者,其病毒载量为 2,000,000 copies/mL,随后接受 23 周的治疗,治疗结束 6 个月内,其病毒载量一直维持在 70 copies/mL 以下。REPLICOR 联合核苷类似物治疗有望降低病毒载量和抑制表面抗原释放,从而使得大量乙肝患者重获有效免疫,控制感染进展,导致持久的病毒学应答。如此,REP 9AC 将成为治疗慢性乙肝的新手段。

六、免疫调节策略

如上所述,通过 PD-1 和 PD-L1 的相互作用下调 CD8 T 细胞功能,可能是导致急性乙肝慢性化的原因[24,33,34,39]。采用特异性抗体阻断这一交互作用可能恢复正常免疫功能。在土拨鼠进行的临床前研究表明,T 细胞功能得以重建。土拨鼠 wPD-1 和 wPD-L1 的这一特性揭示了哺乳动物种间的高度相似性[97]。急性感染期间,CD8 T 细胞表达 wPD-1 与病毒血症和 CD8 T 细胞反应相关[Liu J. and Roggendorf M., unpublished results]。慢性乙肝感染的土拨鼠外周血细胞 wPD-1 表达升高。急性、慢性 WHV 感染动物在体外用抗体阻断 wPD-1/wPD-L1 相互作用可增强淋巴细胞增生,以及 CD107a 的脱颗粒。给予慢性感染的土拨鼠恩替卡韦治疗及 DNA 疫苗(WHcAg 和 WHsAg)反复注射,在疫苗免疫过程中,静脉注射抗 wPD-L1 抗体,结果观察到明显的 T 细胞功能重建。上述结果表明在免疫耐受的形成中,wPD-1/wPD-L 通路扮演了重要角色。在慢性 WHV 携带者的土拨鼠体内,阻断 wPD-1/PD-L 通路,能显著提高 T 细胞功能,打破对病毒抗原的免疫耐受。

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1966 – 1974 年间在波恩大学学习医学。1983 – 1985 年期间就职于美国费城癌症研究所。1985 – 1991 年在慕尼黑大学学习医学微生物学和卫生学,并在此期间担任助理教授和病毒性疾病和肝炎研究诊断实验室主任。1991 – 2001 年间担任德国杜伊斯堡 – 艾森大学病毒研究所主任,主要负责病毒感染和诊断的研究,尤其是肝炎病毒的发病机理和预防机制以及逆转录病毒的感染。目前担任丙型肝炎国家参比中心和狂犬病国家参比实验室主任,是德意志联邦共和国咨询委员会成员和德国病毒诊断委员会主席。

临床研究与新疗法——协作网络的作用

Michael P. Manns

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德国肝脏基金会 Hep-Net

新药获准上市前必须进行一期至三期临床试验,制药行业为此目的建立了自己的协作网络。但是针对罕见病/适应症的新疗法的开发,以及对已批准上市药物治疗方案的优化,研究者发起型临床试验(IIT)和研究者主导型临床试验(IST)是必不可少的。

德国竞争力协作网络 Hep-Net(www. kompetenznetz-hepatitis. de)于 2002 年创建。德国政府(BMBF)在最初 8 年中提供了 1250 万欧元的资助,目前 Hep-Net 由德国肝脏基金会(www. german-liverfoundation. com;www. deutscheleberstiftung. de)主持并资助。Hep-Net 下属项目之一——Hep-Net Study House 主要开展 IIT 临床试验,同时也开展 IST 临床试验,这些试验得到政府、德国肝脏基金会及制药行业的资助。德国 Hep-Net 急性丙型肝炎研究就是其中一项试验。我们建立了一个由 70 多个中心组成的协作网络,对干扰素单一疗法早期干预预防慢性化的效果进行研究。

从图中可见其中包括了大量的慢性乙肝患者,以及丙型肝炎及丁型肝炎患者(图 1,2)。

最近,对使用干扰素单一疗法立即判断和观察治疗后再判断两种策略进行前

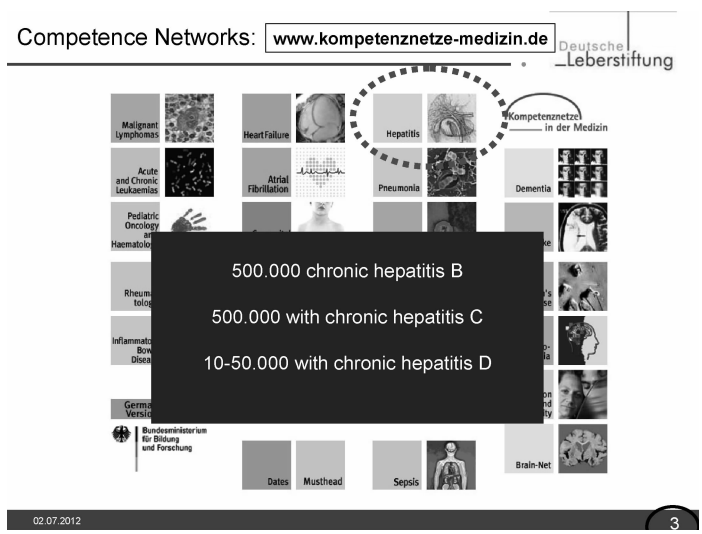


图 1　德国网络的功能

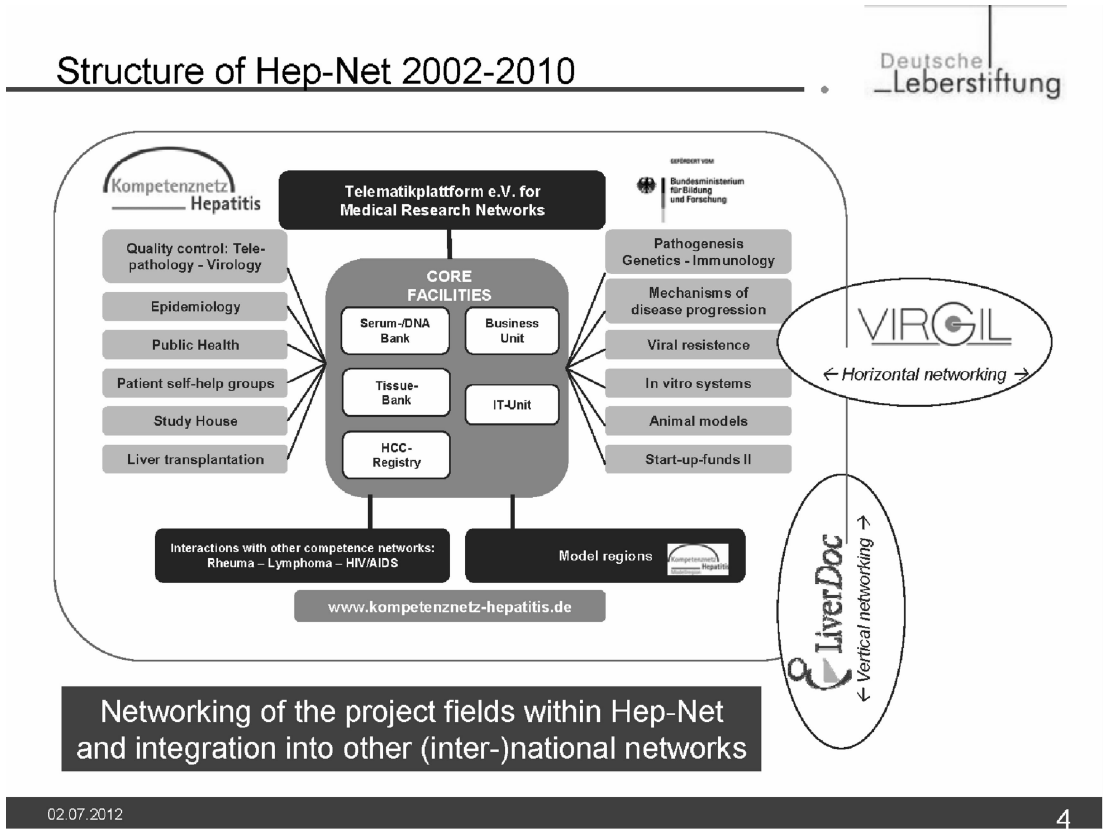


图 2　网络的结构

瞻性比较的 Hep Net Ⅲ期研究已经完成。我们还通过多中心试验对 PEG – IFN 联合利巴韦林的个体化治疗以及根据早期病毒动力学优化丙肝治疗持续时间进行了研究。其中一些研究的结果已被德国以及国际治疗指南采用。此外,我们还与制药企业合作进行审批后承诺研究,例如使用 PEG – IFN 联合利巴韦林治疗 2 型和 3 型 HCV 感染。上述 REDD2,3 研究是第一个将德国协作网络国际化的 Hep Net 研究(见图 3)。

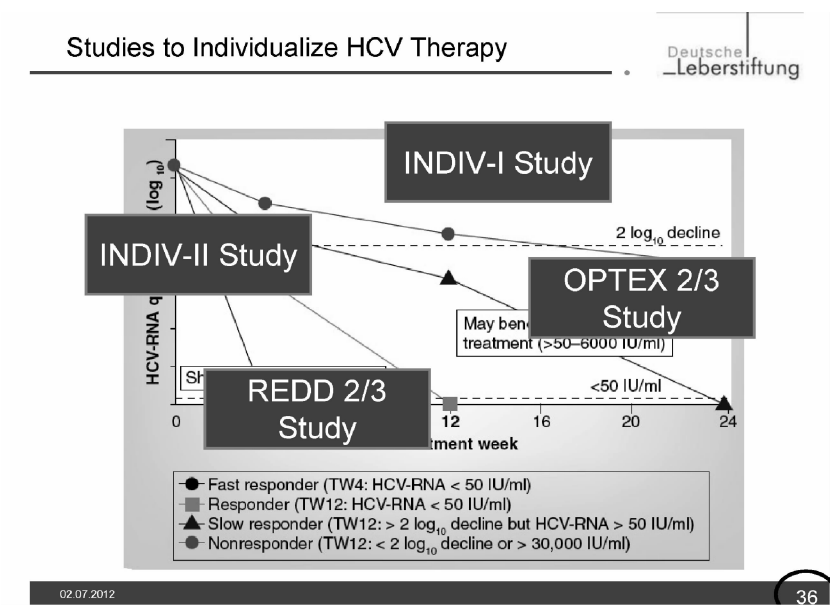
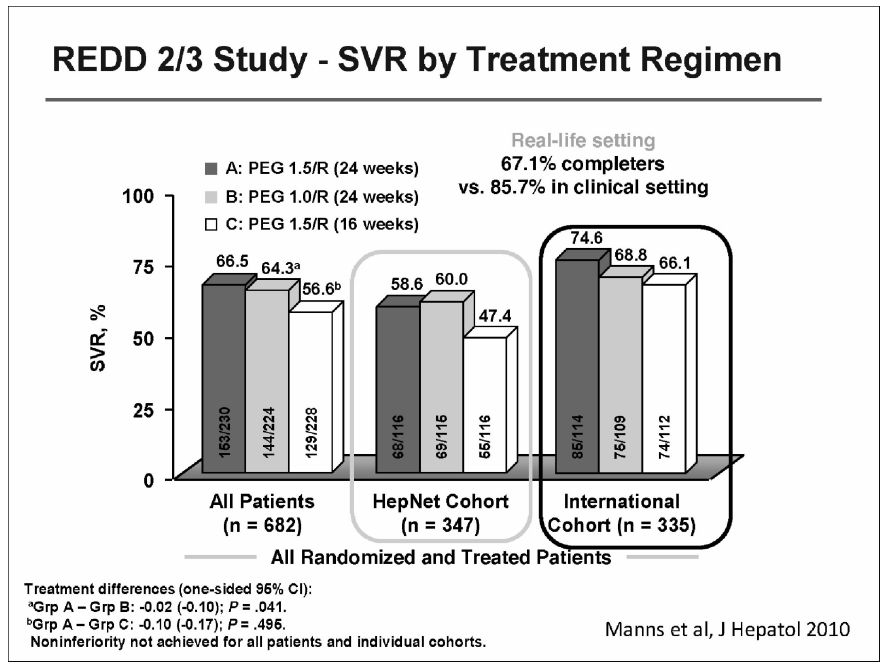


图 3A　丙肝患者个体化治疗的研究设计



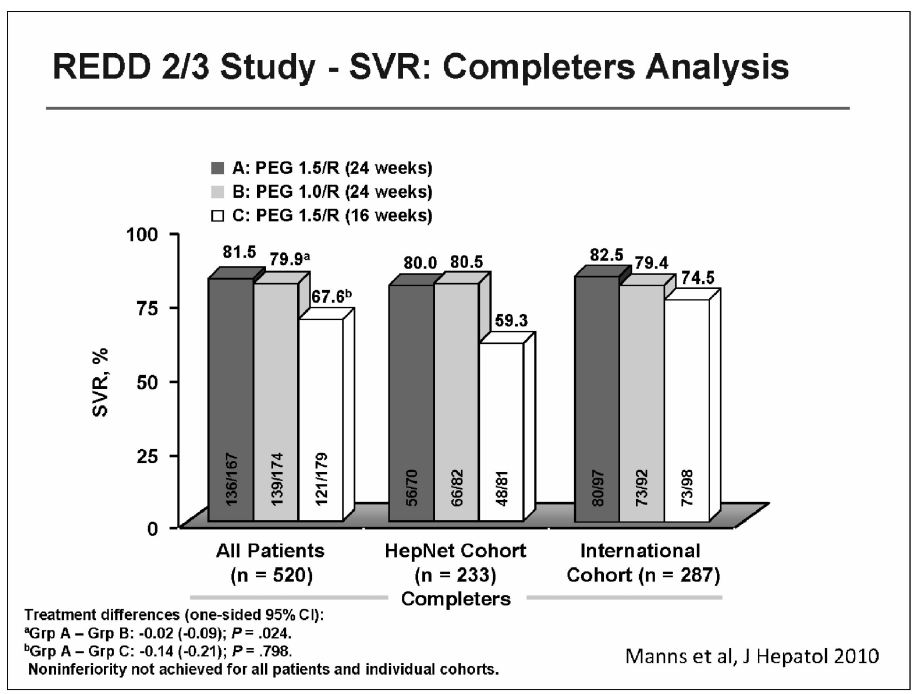


图 3B　用不同治疗方案后的早期病毒应答结果

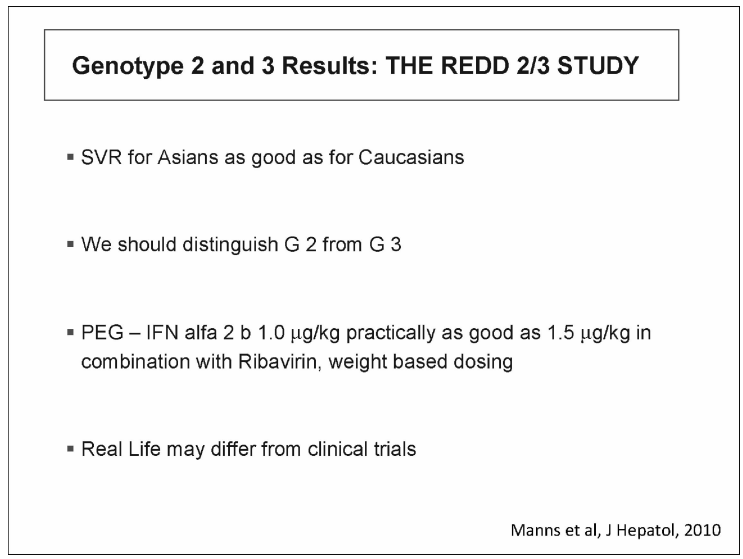
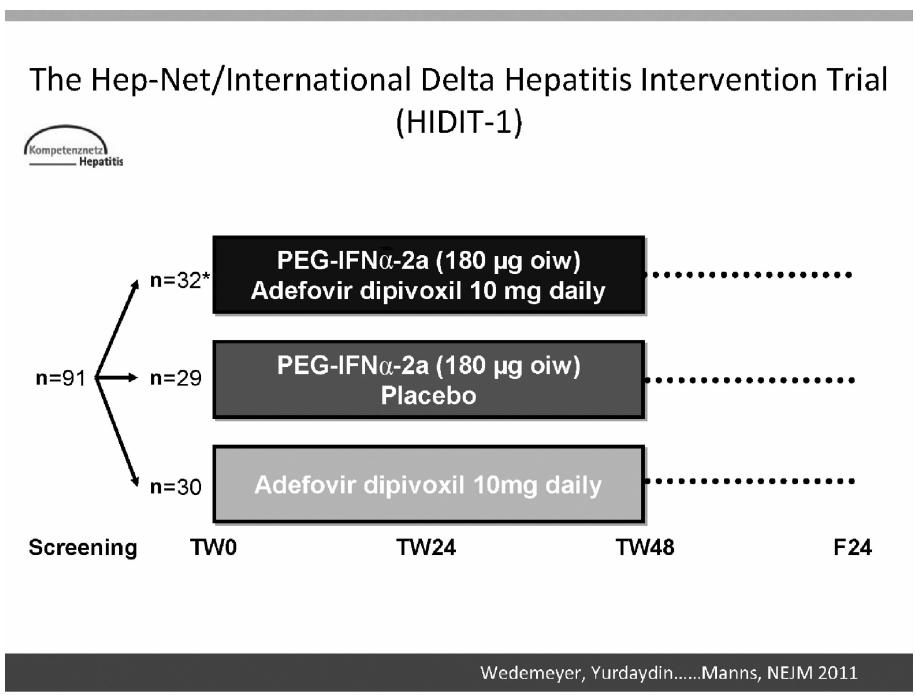
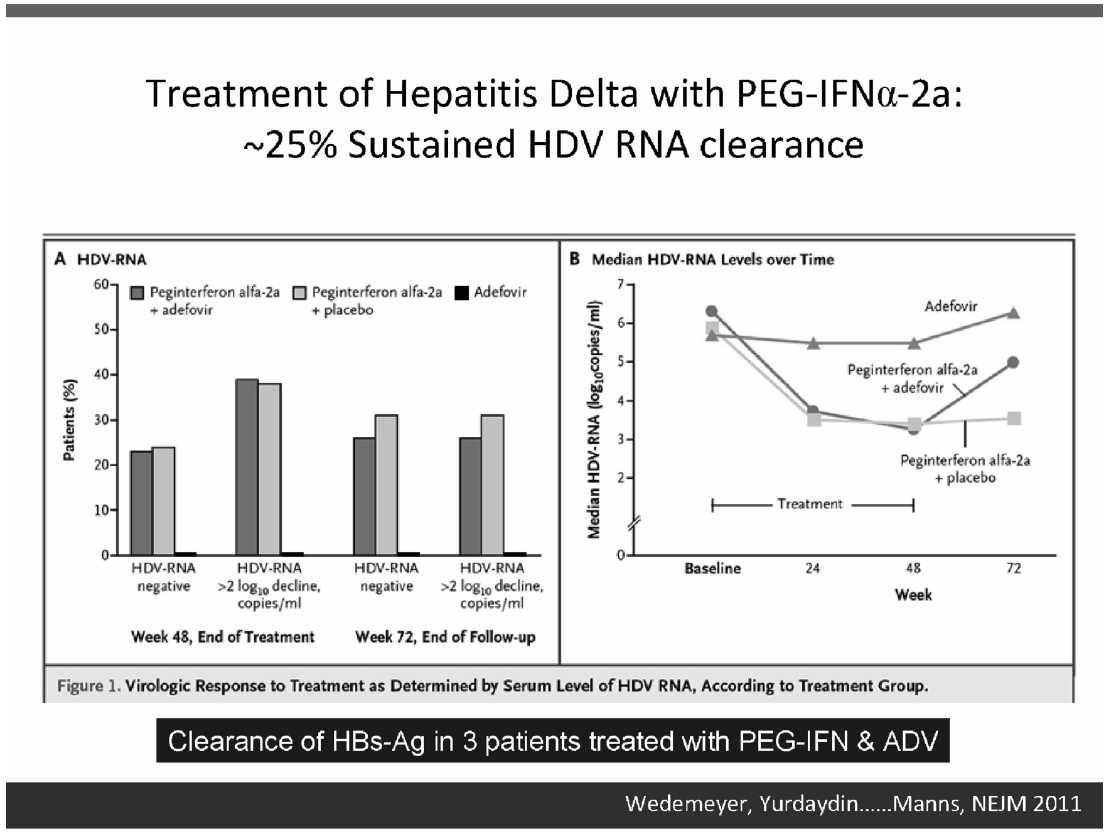


图 3C　丙型肝炎 2 与 3 基因型的治疗结果

最后，Hep Net 还在进行丁型肝炎创新疗法的多国多中心试验。其中第一个试验 HIDIT Ⅰ比较了 48 周 PEG – IFN、阿德福韦及 PEG – IFN 联合阿德福韦治疗的疗效（图 4 – 6）。德国、土耳其和希腊的多个中心参与了该试验。Hep Net HIDIT Ⅱ试验将比较 96 周 PEG – IFNα-2a 单一治疗和 PEG – IFN/替诺福韦联合治疗的疗效。目前 HIDIT Ⅱ试验已完成入组，在土耳其、希腊、罗马尼亚和德国同时进行。Hep Net 研究者还利用上述大型多中心试验数据并借助 Hep Net 科研人员的专业实验技能开展了多个子项目研究，增进了对病毒性肝炎的致病机制和治疗方法的认识。





用 PEG 干扰素治疗丁型肝炎,约 25% 清除 HDV RNA。

用 PEG 干扰素加 ADV,3 个患者清除了 HBsAg。

图 4　用药物干预丁型肝炎的研究方案

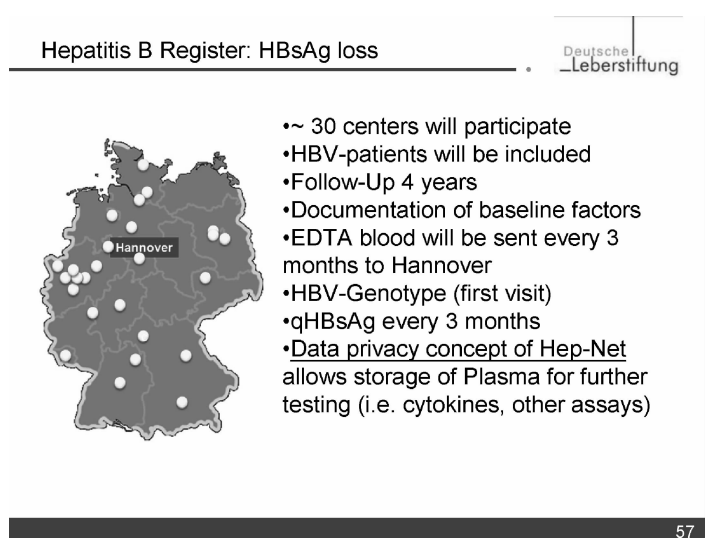
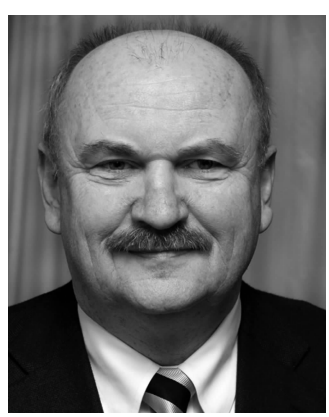


图 5　德国对乙肝患者的 HBsAg 消失的长期随访

(30 个中心参加,随访 4 年)每 3 个月测一次 HBsAg

图 6　德国所有开展感染研究的单位



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1970－1976 年在奥地利维也纳大学医学院、德国美因茨 Johannes-Gutenberg 大学学习，获医学博士学位。1985 年成为胃肠病与肝病专科医师。自 1998 年起担任柏林罗伯特·科赫研究所科学咨询委员会委员。1998 年起担任埃朗根大学医院咨询委员会委员。2002 年创建德国国家病毒性肝炎胜任协作网络 Hep-Net，并担任主席。2006 年创办德国肝脏基金会并担任主席。2006 年担任德国胃肠病学会主席。2011 年，德国内科学会理事会成员。2011 年，德国肝脏病研究学会，下届主席。

主要研究方向为：炎症性肝病，病毒性、自身免疫性肝病，肝病治疗的临床试验，尤其是病毒性肝炎、自身免疫性肝病，肝移植，胃肠肿瘤学，尤其是肝癌，细胞治疗，肝细胞移植，再生医学，粘膜免疫，临床药理学。

中国乙型和丙型肝炎流行情况及对策

王宇

中国疾病预防控制中心

乙型肝炎病毒感染及其相关疾病严重危害中国人群健康,造成严重的疾病负担。乙型肝炎病毒有很高的感染率和病毒携带率,据1992年血清流行病学调查结果,全国人群乙肝表面抗原携带率为9.75%。乙型肝炎疫苗接种能有效地保护受接种者不受乙肝病毒感染。我国自20世纪80年代后期开始使用血源性乙肝疫苗,由于供应量的限制,当时主要在乙肝表面抗原携带率非常高的地区,特别是伴有肝癌高发病率的地区人群中进行接种,使这些地方的人群优先获得了特异性的免疫保护。到20世纪90年代初,酵母来源的基因工程疫苗可以大量供应后,政府推荐和鼓励居民有偿地接种乙肝疫苗。由于受经济条件的限制,导致经济发达地区的城市居民乙肝疫苗接种率高于贫困地区的农村居民。2002年,卫生部将乙肝疫苗纳入国家儿童计划免疫,免费提供疫苗,仅收取少量的服务和耗材费用,乙肝疫苗接种率整体明显上升。2005年,乙肝疫苗接种纳入国家免费的常规免疫规划,根据医疗服务覆盖,使绝大部分新生儿都得到及时的接种。卫生部在2006年组织开展了全国人群乙肝等有关疾病血清流行病学调查。结果显示,全国1~59岁人群乙肝表面抗原携带率为7.18%。1~4岁儿童乙肝表面抗原携带率最低,为0.96%,达到了世界卫生组织西太区制定的乙肝控制目标。15~59岁人群乙肝表面抗原携带率最高,为8.57%。根据1992年和2006年两次血清流行病学调查结果计算,1992年以来,儿童感染乙肝病毒的人数减少了近8000万人,全国至少减少了儿童乙肝表面抗原携带者近1900万人,获得了巨大的社会和经济效益。乙肝疫苗全程接种率由1992年乙肝疫苗纳入计划免疫管理时的30%,提高到2005年出生儿童的93%;乙肝疫苗首针及时接种率由1992年的22%提高到2005年出生儿童的82%,有了大幅度提高。我们可以期望,当目前这一代儿童达到生育年龄时,他们的后代会进入消除乙型肝炎的时代。

虽然疫苗接种将最终消除乙型肝炎病毒感染所导致的多种慢性肝疾患,包括

绝大部分慢性肝炎、肝硬化和肝癌，但这是一个相当缓慢的过程。全人群乙肝表面抗原携带率的下降是靠新生代接种疫苗获得保护，从而可稀释现有病毒携带者而达到明显下降的效果。但是我们不应忽略，在2010年中国仍然有乙肝表面抗原携带者约9300万人，其中当年慢性肝炎约3000万，肝硬化约100万，肝癌约35万。这种状况还将持续多年，成为我国严重的疾病负担。

一般情况下，乙肝表面抗原携带者体内病毒浓度与肝损伤程度成正相关。在没有直接、彻底清除体内病毒的治疗方法时，能降低血液中乙肝病毒载量水平的抗乙肝病毒药物就成为首选。迄今，抗乙肝病毒药物有了很好的发展，在临床应用的有7~8种之多。由于不同的药物获得的治疗效果不同，治疗成本也有很大差别。对于巨大的、需要长期治疗的患者人群，我国应更好地组织针对慢性肝炎的抗病毒治疗方案研究，建立循证的（Evidence-Based）临床治疗方案并加大对临床治疗的指导，会非常有利于提高疗效、降低费用，取得优化的综合效益。

丙型肝炎病毒的传播，主要途径之一是医源性感染。我国自上世纪90年代中期广泛进行采供血严格筛选以来，丙型肝炎病毒新发感染已很少。然而，在注射毒品人群中，同时感染HIV、乙肝与丙肝者的新发丙型肝炎者人数在增加。此外，因缺乏教育与管理，不洁医疗行为传播丙肝病毒感染的风险也大为增加。近期在我国河南、安徽、广东等地农村陆续发现了聚集性丙肝病毒感染病例，其中儿童、少年占很大比例，说明新发感染病例增多，提示原已日趋减少的医源性感染又有抬头。为了减少丙肝病毒的医源性感染，应不失时机地将防控重点放在基层医疗机构，改善和提高人员素质，加大控制力度，通过培训和指导，减少和避免在一般人群中发生医源性感染。

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主要科研领域：乙肝病毒分子生物学。乙型肝炎发病机制及乙肝诊断试剂的研究，丙型肝炎病毒基因分子生物学，丙型肝炎基因工程疫苗，HGV/GBV 的分子生物学研究及肝癌抗原肽及肝癌免疫治疗等。

曾获卫生部科技进步一等奖（中国大陆丙型肝炎病毒基因分型的研究）。茅以升北京青年科技奖，国家教委优秀青年教师基金，“做出突出贡献的中国博士学位获得者”称号及”全国卫生援藏工作先进个人荣誉称号”。

病毒性肝炎疫苗研发的未来

阮力

中国疾病预防控制中心病毒病预防控制所

病毒性肝炎是一类以肝细胞为主要宿主的病毒引起的肝炎。这类病毒主要包括甲型肝炎病毒(HAV)、乙型肝炎病毒(HBV)、丙型肝炎病毒(HCV)、丁型肝炎病毒(HDV)、戊型肝炎病毒(HEV)等。其中,从公共卫生角度考虑,需要使用疫苗来预防和控制的肝炎主要有四种:甲型肝炎、乙型肝炎、丙型肝炎和戊型肝炎。这些疫苗的研发包括两方面:预防性疫苗和治疗性疫苗,前者上述四种肝炎都需要,后者主要集中在乙型肝炎和丙型肝炎。目前,预防性甲型肝炎、乙型肝炎和戊型肝炎的疫苗已研制成功,丙型肝炎疫苗已进行了Ⅰ期临床研究,治疗性乙型肝炎疫苗已进行了Ⅱ~Ⅲ期临床研究,丙型肝炎的治疗性疫苗正在进入Ⅰ~Ⅱ期临床研究。从病毒性肝炎防治需求来看,需要哪些疫苗?哪些疫苗是急需的?应采取哪些相应对策?笔者将从病毒性肝炎疫苗的研发现状、存在问题、发展趋势及相应对策等方面,提出以下意见供大家讨论。

(一)甲型肝炎疫苗

甲型肝炎由甲型肝炎病毒引起,临床以典型急性黄疸性肝炎为特征。甲型肝炎通常能痊愈,不会转变成慢性。儿童多为亚临床感染,成人感染多为急性肝炎。甲型肝炎病毒属小 RNA 病毒,主要通过粪口途径传播,传染性较强。保护性粘膜免疫和血清中和抗体都能阻断病毒进入肝脏细胞,相应细胞免疫能有效清除感染病毒,促进疾病恢复。

1)疫苗需求　预防性疫苗是预防和控制该病最有效手段,不需要治疗性疫苗。

2)疫苗现状　主要有两类:一为灭活疫苗,由野毒株灭活后制备而成;二为减毒活疫苗,由野毒株在细胞中传代减毒后制备而成。

3)存在问题　灭活疫苗:高度安全,免疫效果好,但价格相对较贵,使用野毒株(或部分减毒株)制备灭活疫苗在生产过程存在生物安全隐患。减毒活疫苗:

安全,免疫效果好,价格相对便宜,贮存运输较复杂,毒株存在重组和反祖隐患。

4）应对策略 目前,两类疫苗基本满足甲型肝炎防控需要,但随着社会的进步和经济的发展,甲肝疫苗尚需进一步的改进。① 分子减毒活疫苗的研发,精确去除毒力相关基因,解决反祖问题;② 使用稳定减毒疫苗株制备灭活疫苗,在解决减毒活疫苗反祖问题的同时,解决生产过程中操作野毒株引起的生物安全隐患。③ 研制基因工程 VLP 亚单位疫苗,替代灭活和减毒活疫苗。④ 研制含甲肝的合理的多价联合疫苗,如:多价灭活疫苗、多价减毒活疫苗、多价亚单位疫苗等。

（二）乙型肝炎疫苗

乙型肝炎由乙型肝炎病毒引起,临床以非典型黄疸性肝炎为特征。婴幼儿感染乙型肝炎病毒多数出现长期带毒,相当一部分带毒者出现慢性肝炎,成人感染多数痊愈。乙肝病毒为嗜肝 DNA 病毒,病毒在环境中比较稳定,主要通过血液、性交及母婴途径传播,传染性较强。血清中和抗体能阻断病毒感染肝脏细胞,相应细胞免疫能清除感染病毒,促进病人恢复。

1）疫苗需求 预防性疫苗是预防和控制该病最有效的手段,治疗性疫苗将为慢性乙肝病人康复带来希望。

2）疫苗现状 ① 预防性疫苗已获成功,基因工程乙肝疫苗(酵母和 CHO 细胞生产)已取代了血源乙肝疫苗广泛用于该病的预防;② 治疗性疫苗:在中国,已有三种疫苗进行了临床研究,一是抗原抗体复合物疫苗(已完成 IIIa 期),二是合成肽疫苗(已完成 IIa 期),三是 DNA 疫苗(已完成Ⅰ期)。

3）存在问题 ① 预防性疫苗:安全性高,效果好,成本低,但有 3% ~10% 左右接种者对该疫苗呈无反应或低反应;② 治疗性疫苗:临床效果尚不理想,临床研究的疫苗较少,总共 3 个(在中国),其中两个进入Ⅱ ~ Ⅲ期。

4）应对策略 ① 目前,预防性疫苗基本满足社会需求,从市场考虑,需解决低反应或无反应人群的疫苗接种问题。开发大剂量(60 μg/剂)乙肝疫苗,或带 PreS1 等多种抗原的乙肝疫苗是解决此问题的重要途径;② 在单价乙肝疫苗用于母婴阻断的同时,乙肝疫苗可与其他蛋白疫苗组成多联多价疫苗,用于非母婴阻断的免疫;③ 治疗性疫苗具有社会需求和市场需求的迫切性,但目前仍处于概念性验证阶段,应加强打破乙肝免疫耐受问题的基础研究;鼓励不同思路和设计方案的临床研究尽快进入Ⅰ~Ⅱ期临床;④ 进行治疗性乙肝疫苗与治疗性药物联合应用研究。

（三）丙型肝炎疫苗

丙型肝炎由丙型肝炎病毒引起,急性感染病人多无严重临床症状和明显黄

疸。急性感染丙型肝炎病毒的病人中,20%左右会彻底清除体内病毒而痊愈,多数病人转为慢性感染,是肝癌的重要病因之一。丙肝病毒为单股正链 RNA 病毒,属黄热病毒,通过血液、性交及母婴等途径传播。虽然丙肝病毒急性感染者中有20%左右的病人可以痊愈,提示研制成功丙肝疫苗的可能性,但丙肝病毒的高度变异性和多数感染者转为慢性,预示了丙肝疫苗研制会极为困难。

1)疫苗需求 ① 在治疗性药物取得极好疗效的今天,一个安全有效的丙肝疫苗,对控制丙肝病毒感染,特别是高危人群感染仍具有重要意义;② 同样,治疗性疫苗可能在提高药物治疗效果、降低药物治疗成本及应对可能出现的病毒耐药性均具有实际意义。

2)现有疫苗 尚无疫苗上市,预防性病毒载体疫苗、VLP 疫苗进行了Ⅰ期临床研究;治疗性疫苗已处于临床Ⅰ~Ⅱ期研究阶段。

3)存在问题 预防性和治疗性疫苗仍处于概念研究阶段,一些动物实验提示了一定的免疫保护,但整体的研发路线尚未走通。

4)应对策略 ① 预防性疫苗:加强自然感染恢复者中免疫保护机理的研究;由于丙肝病毒高度变异,丙肝疫苗本质上应是广谱疫苗,确认广谱交叉中和抗体和广谱交叉细胞免疫的研究尤为重要。② 治疗性丙肝疫苗:加强打破丙肝病毒慢性感染的基础研究;鼓励治疗性疫苗研究尽快进入Ⅰ~Ⅱ期临床;重视治疗性疫苗与治疗性药物联合应用的研究。③ 理论创新:丙肝病毒高度变异,并引起慢性感染,按传统疫苗研制理论和策略很难取得成功,理论创新对研制如丙肝一类疾病的疫苗尤为重要。

(四)戊型肝炎疫苗

戊型肝炎由戊型肝炎病毒引起,以急性黄疸性肝炎为其主要临床特征。戊型肝炎病毒主要通过肠道传播,亦可通过输血和垂直传播。全球约三分之一人口曾感染过戊肝病毒,但通常为亚临床感染,病例多呈散发,也可引起暴发流行,急性感染多能痊愈。戊肝病毒为单股正链 RNA 病毒,属戊肝病毒科戊肝病毒属成员,只有一个血清型。虽然戊肝病毒有多种动物感染模型,但尚不能在体外大量培养,因此,使用基因工程技术是该疫苗研制的主要途径。血清中和抗体和粘膜免疫都能阻断戊肝病毒对肝细胞的感染。

1)疫苗需求 从疾病预防和控制角度来看,戊肝疫苗具有重要意义,戊肝不需要治疗性疫苗。

2)疫苗现状 使用大肠杆菌表达 HEV ORF2 片段形成的 VLP 制备的疫苗完成了Ⅲ期临床研究,有较好的保护效果,已于 2011 年 12 月批准在中国生产上市。

3)存在问题 疫苗刚上市,大规模应用的情况尚待分析研究。

4）应对策略　① 在高发地区推广使用，进一步观察免疫效果及安全性，并对其成本效益进行分析研究。② 进行含戊肝疫苗的多联多价亚单位疫苗的研究，以推动戊肝疫苗的应用。

病毒性肝炎疫苗的研发是近 30 年来传统疫苗和基因工程疫苗研发的一个缩影。一方面它反映了传统疫苗技术在疫苗研发中的价值和地位，例如甲肝减毒和灭活疫苗及乙肝血源疫苗等；另一方面也反映出基因工程疫苗在改造传统疫苗和研制新型疫苗中不可替代的作用，例如：基因工程乙肝疫苗是使用基因工程技术改造或替代传统疫苗（血源乙肝疫苗）的典范，而戊肝疫苗则是使用基因工程技术研制成功了传统技术无法研制的新型疫苗的代表；丙型肝炎疫苗及乙肝治疗性疫苗的研究则反映了只有基因工程技术才能给那些型多易变并引起持续性感染的病毒疫苗研究带来希望。

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阮力：博士，研究员，曾任中国疾病预防控制中心病毒病预防控制所所长，病毒病应急技术中心主任。1980 年以来，主要从事病毒遗传变异与免疫的研究，并在此基础上进行病毒诊断、基因工程疫苗等生物技术的研究与开发。近年来曾负责 SARS 监测检测及天花、猴痘等预防控制工作。承担过国家科技攻关、863 计划、科技重大专项、自然科学基金，美国 NIH、欧盟及卫生部等 20 多个研究项目。先后建立了哺乳动物细胞、重组痘苗病毒等基因表达系统，研制成功乙肝基因工程疫苗，5 个重组痘苗病毒疫苗进行了Ⅰ期临床研究。1986 年在美国获纽约西奈山医学院杰出客座科学家证书，1991 年获国家做出突出贡献的优秀留学回国人员证书，1991 年获政府特殊津贴，1995 年获国家杰出青年科学基金，1996 年获国家突出贡献专家称号。1993 年乙肝基因工程疫苗研制获国家科技进步一等奖，1997 年天坛株痘苗病毒高效表达载体研究及应用获国家科技进步二等奖，另获省部级奖四项。负责和参与 20 余部著作编写，申请专利 11 项，发表论文近 200 篇。

迫切需要用疫苗、新型治疗策略和公众教育以控制丙型肝炎病毒感染

Michael Houghton

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据世界卫生组织(WHO)估计,全球丙型肝炎病毒(HCV)携带者已经达到1.7亿,且每年有数百万的新增感染出现。HCV 感染者经过数十年的病程,约 20% 可能发展成为肝硬化,而最终约有 5% 的感染者发展为肝癌。因此,中国等 HCV 高度流行的国家目前迫切需要能有效控制 HCV 传播的策略来减少这一疾病带来的危害[1]。首先,必须对公众进行普及教育,避免 HCV 通过血液传播,例如杜绝使用不洁注射器和输液管,杜绝纹身或者打耳洞时使用不洁针具,杜绝一些可能造成病毒经血液传播的传统医学手段,杜绝其他一些可能的血液传播方式。其次,改善对患者的治疗,在北美新型 HCV 蛋白酶抑制剂已经于 2011 年获批上市,与干扰素和利巴韦林联合使用,可以治愈约 70% 的感染者,疗效显著[2]。这些新型药物的推广使用显然刻不容缓。不少抑制 HCV 多聚酶、NS5A 以及其他靶蛋白的新药也正在进行临床试验。当这些药物与已有的治疗手段相结合,完全有可能治愈所有的 HCV 感染者[2]。与艾滋病病毒 HIV 治疗相同,联合用药还可以避免病毒出现耐药性[2]。但不同药物搭配治疗价格昂贵,因此各国政府必须在经济和政策上做好准备。有效的治疗还包括对病人进行精细的临床管理,包括病毒耐药监测。在加拿大等地,人们在互联网上建立临床论坛,互相分享重要的信息并提供专业的建议,有效地打破了地域局限。使用溶瘤病毒治疗肝癌是另一个具有巨大潜力的新领域。目前对肝癌治疗的方法仍然非常有限,而利用减毒病毒特异性定位并杀死肿瘤细胞的策略,开辟了一个重要的医学研究领域。最近,在美国的一项二期临床试验报道,利用过去用于制备天花疫苗的一种容易生长的减毒牛痘病毒多次给药的方法,使肝癌患者的无恶化生存时间延长了一倍[3]。而其他一些利用溶瘤病毒治疗肝癌和其他癌症的研究,也处于临床开发阶段,前景广阔。

尽管过去人们普遍认为 HCV 疫苗研究前景悲观,但目前看到了一些新的值

得欣喜的希望。我们现在知道,有针对 HCV 的天然免疫存在;这种免疫反应不仅与针对病毒的保护性 CD4 + 和 CD8 + T 细胞相关,同时与早期产生的中和不同病毒的交叉抗体相关。因此,在动物试验中,先用疫苗免疫动物,再感染 HCV 病毒,可以显著降低 HCV 感染慢性化的比例[4]。一个基于缺陷型腺病毒的预防性疫苗正在美国进行二期临床试验,这种腺病毒可以表达 1b 亚型(在中国流行最广泛的病毒亚型)HCV 病毒的 NS3/4/5 多种非结构蛋白[5]。这种疫苗仅具备部分有效的保护性,但它的意义在于可能产生针对多种其他型别 HCV 的交叉中和抗体。我们实验室最近证明了在疫苗接种者体内确实存在这样的交叉免疫反应。最后,在 2012 年 EASL 会议上来自 Transgene 及其合作者的报道指出,当 HCV 疫苗与抗病毒的干扰素和利巴韦林联合使用时,具有一定的治疗价值。经过 12 周的治疗,在疫苗联合抗病毒药物组中病毒清除的人数是单用抗病毒药物组的两倍[6]。这种疫苗的生产也非常简单,即利用缺陷型牛痘病毒表达 HCV 非结构蛋白 NS3/4/5。

总之,控制 HCV 感染已经取得了长足的进展。这主要依赖以下的策略:1)普及公众对 HCV 传播途径的认知;2)新型 HCV 蛋白酶、聚合酶等病毒蛋白抑制剂以及治疗性疫苗,同现有治疗手段的联合使用;3)能激活针对不同型别 HCV 病毒的细胞免疫及中和抗体反应的预防性疫苗的使用。

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Michael Houghton 教授现任加拿大阿尔伯塔大学李嘉诚病毒学研究院教授,是李嘉诚教授和病毒学领域加拿大首席科学家的获得者。主要从事病毒性肝炎等传染病的研究。是丙型肝炎病毒的发现者之一。并对丙型肝炎病毒进行了大量血液检测,药靶研究和临床疫苗研究。同时他的实验室还最早确定了 HDV 病毒和人 β - 干扰素基因的分子性能。因在 HCV 研究方面做出的贡献,Michael Houghton 教授和他的同事们获得了包括美国临床 Lasker 奖,Karl Landsteiner 奖,德国 Robert Koch 奖以及加拿大肝病学会金奖等奖项。Michael Houghton 教授已先后发表论文 200 余篇。

未来影响肝病临床与流行病学发展的趋势

F. Blaine Hollinger

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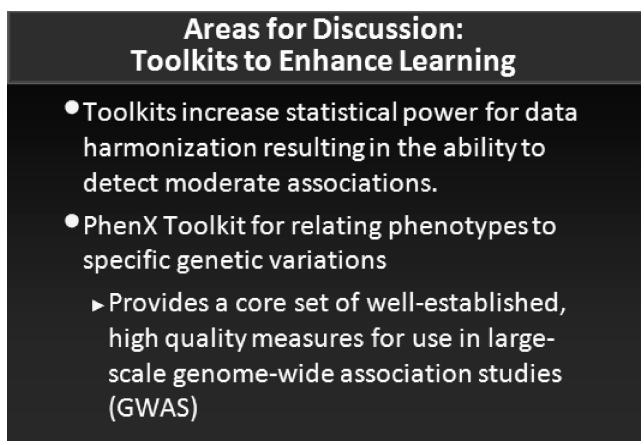
本文主要讨论在未来20年来会影响肝病临床及流行病学的一些因素。预测未来20年,是一个难题,正如中国古代诗人老子在公元前6世纪曾说过“知者不博,博者不知”。预测未来可能对,也可能错。有些学者认为未来与过去完全一样,只是经济开支会更贵。下面就三方面进行讨论,或许有益。

第一,很显然,肝病学家与胃肠疾病专家肯定不能处理全部肝病患者,而需要与非专家如家庭医生、内科医生或护理人员共同发挥作用,这一客观现实将对卫生与经济产生重大的社会影响,并将避免未治疗疾病带来的严重后果。总的目标是,教育和帮助初级医务人员通过运用视频、电话会议、智能手机及网络,从而使他们会诊断和处理无并发症的比较简单的肝病,这样可以改善患者的预后。在通过共识建立的指南的基础上,分享最好的医疗实践经验,从而减少对病人处理上的差异。其使命在于扩大在医疗服务欠发达地区对肝脏病人的关照,通过基层医务人员和肝病学家的不断地互动,监测病人病情发展的后果。可以期望,通过这种方法,对医疗体系的益处将是巨大的。通过网络,可减少各种处理或治疗方法的差异,并及时传播和强化有关的知识。每周七天,每天24小时的实时会诊为该项目提供基本的框架,通过由肝病学家、放射学家、肿瘤学家一起开展每周一次的电话医学门诊,来讨论病例,教育和指导社区医生。职业上的双向交流与反馈可以使专家与基层工作者均得益,这样可以避免过多的检测和旅途费用,对病人的处理也可减少费用。对于这一项目的有效性,通过建立一个符合HIPAA的、基于网络的数据库进行评估。只有当病情非常复杂时,方需将患者转诊给专家。

在美国,该项目已取得了一些成功。该项目称为ECHO(社区医保的延伸),是新墨西哥州Albuquerque新墨西哥大学卫生科学中心的Dr. S. Arora创建的。这样一个模型可使在任何地区居住的重病患者获得高质量的医疗服务。ECHO已使数以千计的病人获得医疗,其中多数来自偏远和贫困地区。初级医务人员可

讨论内容:增强学习的工具包

为了更进一步了解和治疗肝病,应设计工具包或工具书,提供循序渐进的指南和其他资源,帮助医生、科学家及公共卫生工作人员针对公共卫生问题开展持续的、及时的、和恰当的调研。这些工具包或书有别于教科书,它们有详尽的线路图和详尽的阐述。



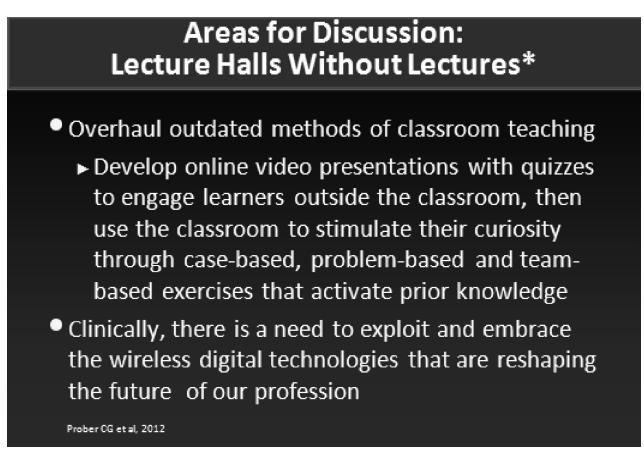
讨论内容:增强学习的工具包

工具包增强了对数据整合的统计学处理能力,因此可以检测出一些微弱的相关性。PhenX 工具包可将表型和基因型的变异进行相关性研究。为大规模、基因组范围的相关性研究(GWAS)提供一整套成熟的、高质量的方法。

获得如何处理复杂肝病病人的指导,许多病人寿命延长,疼痛和失能减少,更加健康。一个治疗基于社区的慢性丙型肝炎病人的试验已经展开。初步结果显示由社区医生处理的慢性丙肝病人的治疗质量与 SVR(长期病毒应答者)与大学医院没有显著差别,病情较复杂的病人则转由专家处理。这一创新模式具有减少经济开支,同时维持或增强医疗质量的潜力。但关键问题是如何使医疗企业,从医学和经济学的角度,相信这一模式的价值;而这也是争论的焦点所在。这一模式是否能使病人获得经专家诊治同样质量的医疗服务?答案可能是不。但是,病人对这一模式的适应性和接受性,和在早期及时发现病人并在出现不可逆的并发症之

前给予治疗肯定是应用这一模式最有利的效果。

另一个现在与将来肝病专家面临的挑战是构建一个恰当的学习肝病临床与流行病学特征的环境。针对疾病的处理和流行病学研究,可以设计与实施 toolkit(工具简易盒或“建议套餐“),从而使资料不会被丢失或篡改。常常碰到这样的情况,在对某个题目进行荟萃分析时,不同的研究无法通过合理的方法整合在一起,许多研究必须进行审查。举一个例子,来说明工具包是如何帮助由许多独立的研究者开展的研究项目。如 Phex 工具包,用来比较各个研究之间表型和暴露。该工具包为大规模基因组学研究提供了一套完善、低负担、高质量的核心方法[5,6]。上述项目是基因组学技术快速发展的产物,这些技术的发展导致出现众多的基因组学相关的研究(GWAS),目的是将表型与特异的基因变异相关联。通过分析数以千计的基因多态性可以使我们进一步了解病因、病理过程从而开发新的预防或治疗药物。为了促进各个研究间快速和有效的评估,位于北卡罗那州研究三角州公园的国际性 RTI 与国家人类基因组研究院(NGRI)正与各个领域的活跃的专家,包括来自科学界的专家采用一个统一的方法,开展合作。RTI 是独立的非营利性组织,其使命在于将知识转变为实践,从而改善人类状况。通过广泛应用这些方法,可以更快地获得统计数据,从而获得有意义的结果。这一共用的 toolkit 还将包括对环境暴露的因素(环境、个人行为、治疗方法),通过共有的方式,获得结果,共享材料,以后再作大量荟萃分析(meta-analyses),将会发现新的基因相关性,值得一提的是胃肠疾病传染病与免疫学占这一项目中 20 个重要内容中的 2 个。



演讲但没有课堂

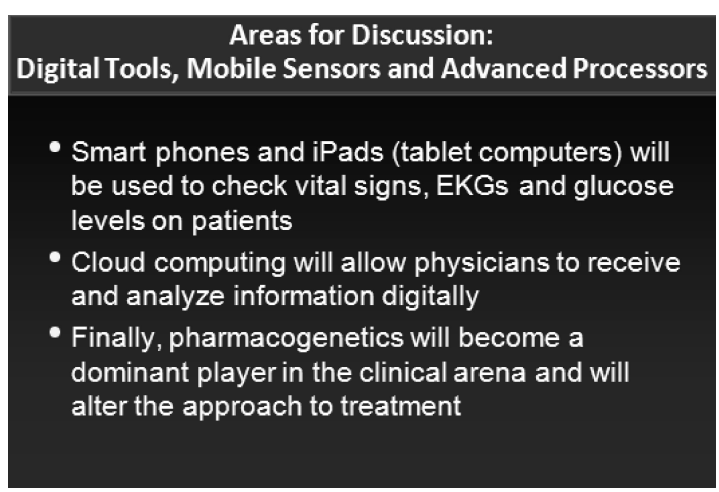
应该放弃使用已过时的课堂教育,发展在线视频,并提出考试题。然后用课堂教育来激活学员们的好奇心;通过病例,提出问题,发挥集体的努力来激活学员们主动地获取知识。

临床上,需要运用并发展无线数字技术,使学生们了解未来职业的需求。

有一个组织是免费可以获得公共社区材料的组织(NACCHO)[7]。其中一个工具盒(书)是关于成年人预防注射乙肝疫苗的内容[8]。另外还有单位建立了现场流行病学的工具盒(toolkits)[9],一步步地指导如何进行现场工作,从而可对传染病的爆发及时了解。此外 WHO 还有一个非常好的工具盒,指导如何安全地进行注射[10],并指导在不同条件下该如何进行及实施。另外还有"循证医学"的工具盒等[11,12,14],对可能由于输血引起传染病也有简易指导的工具盒[13]。可以预测在未来几年,这种工具盒将会大量出现。因为不同于教科书或路径图,更简便更有指导意义地帮助基层医疗及卫生工作者,并可更好地处理肝病患者。

除上述工具盒外,肝病学家、统计学家和流行病学专家将可更好地开设更深入的课程或讲学,如美国和欧洲肝病学会都曾举办过这些讲座。应该将这些内容放在网络上传播,有利于全球肝病工作者均可获得有关专业知识。此外应该建立一个中央网站并由政府支付费用,通过该网站,由肝病专家用其临床经验利用 Skype、email 或电话会议来传播知识,应该要有实时,每周 7 天,每天 24 小时的服务,并由法律保护。

肝病学的未来正在发生巨变,过时的课堂教育将被采用新技术的教育模式所取代。应该考虑发展一些 10 ~ 15 分钟的视频短片。并提出测验题,使得课堂外的学生可以自学,然后用课堂来回答他们那些好奇的问题。通过基于病例、问题的系列问题可以激活他们对知识的探索[15],这种模式或将会使学生对教育有"饥饿感"。临床上我们需要探索无限联网的利用,并用新技术来重塑我们职业的未来[16]。云计算将容许使用者扩大其知识容量与能量。这些超级计算机将容许参与其中的会员间共享知识及技能。



数码工具、移动信息和先进的处理器

智能手机或平板电脑将能用于测定生命体征、心电图和血糖水平

云计算将容许内外科医生获得信息

最后,药物基因组学将改变临床治疗的途径。

下一个时代的医生将采用数码工具,即用移动信息和先进的处理器,从而完全转换我们对个体的了解。智能手机将用于测定生命体征,了解心脏节律及血糖水平等;远程医生可以用超声技术等了解病情,并通过无线网络传递出去;植入物的信号等可以被编码,可以在血液中检测肿瘤细胞。由于加入这些信息系统等,可出现医生—患者间一种新型的合作关系。药物基因学家将在临床工作中起重要的作用。应用各种药物及患者的变异基因测定可能发生的对药物的副反应,如发现 IL-28B 的多态性帮助肝病学家可预测丙肝患者对药物的应答。医疗保险单位将非常关注用基因测定是否对一些治疗发生应答,如 Sam Waksal 原来是 ImClone 公司的 CEO,曾因内部交易入狱 5 年,现在是 Kadmon 公司的 CEO,提出一个"对应答模式资助"的计划,即只对病人对药物有应答者才支付费用[17]。他认为建立一些预测是否反应的方法可以大大节约医疗费用。这些可能是生物技术的未来,但不会在一夜之间发生。总之,个体化治疗和靶向治疗将改变医生对患者的治疗方法。

本文试图探索遗传学与生物学的进展与新发现将如何对医学产生革命性的影响以及临床与流行病学领域的肝病学家如何利用这些新发明。我们应接受新技术并寻找新的方法对多种资料更有效地分析,从而能更好地为病人服务。

肝病临床及流行病学世界趋势

利用少量医疗资源扩大为未获得优良服务的人群的服务。

通过工具盒促进教育,淘汰落后教育模式

采用创新性无线技术加强好的临床实践。

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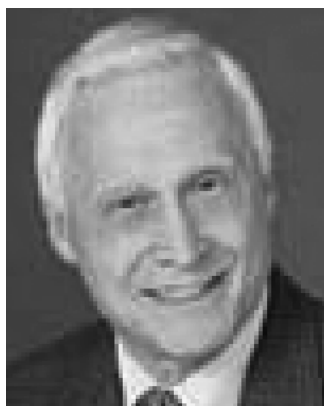

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教授在美国堪萨斯大学获得医学博士学位,并先后在美国加利福尼亚大学医院,美国华盛顿大学附属医院,美国堪萨斯大学医学中心和美国贝勒医学院病毒和流行病学系进修。Hollinger 教授的主要研究方向包括:病毒性肝炎、自身免疫性肝炎、药物性肝炎、原发性胆汁性肝硬化、代谢性肝疾病、酒精肝、血源性致病体、病毒性肝炎的免疫发病机理、早期丙肝感染和病毒结构,在培养液和小动物模型中的丙肝病毒复制,美国血液供应安全,及隐匿性乙肝与丙肝。

Summary of the Top-level Forum

Viral Hepatitis and Liver Cancer—Two Decades Ahead

Yumei Wen

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1. Background

Viral hepatitis, liver cirrhosis and liver cancer are a group of diseases that rank the most important medical and health problems for Chinese people. To date, viral hepatitis can be caused by hepatitis A, B, C, D and E viruses. Hepatitis A and E are transmitted by fecal-oral route through contaminated food or water, and usually do not progress to chronic hepatitis, liver cirrhosis or liver cancer. Although hepatitis A caused a huge outbreak in Shanghai back in 1988, since the use of preventive vaccine, the incidence of hepatitis A has significantly decreased. Recently, individual chronic hepatitis E cases have been reported in patients who are immuno-compromised. However, no liver cancer has been associated with hepatitis E infections. Hepatitis B and C are transmitted by contaminated blood or blood products, and also by mother-to-infant transmission or sexual transmission. Liver cirrhosis and liver cancer is serious sequelae of these infections. Hepatitis D virus can only replicate with hepatitis B virus, and co-infections of these two viruses are rarely found in China. According to the published data from Chinese Ministry of Health in 2011, the incidence of viral hepatitis in mainland China was 102.34/100,000; and the number of cases was 1,372,344, both listed as the top among all 28 mandatory reporting infectious diseases. Furthermore, viral hepatitis B was the first among all viral hepatitis cases. Liver cancer ranked the second high among all cancers in males, and the third in females. Thus, basic and applied studies to control hepatitis B, C and liver cancer are urgent needs.

As early as 1992, hepatitis B vaccination was recommended among newborns and

in 2002, hepatitis B vaccination was included in the expanded program of immunization in China, and since 2005 a universal vaccination of newborns with hepatitis B vaccine has been free of charge in China. These have brought significant success in decreasing the carrier rate of hepatitis B surface antigen (HBsAg) in the population, which was announced as 7.18% in a recent nation-wide survey. However, there are still around 3% of the vaccinees who are low or non-responders to the vaccine. Currently, it is estimated that there are 93 millions of HBsAg carriers in China, and 10 millions of hepatitis C infected persons. These two viruses can cause chronic infections and liver cancer, and there is still no effective method to cure HBV infections. In the national key scientific programs of the 11th five-year plan, and the 12th five year plan, research and control of viral hepatitis B and liver cancer have been listed as important projects, especially the reduction of their incidences and morbilities.

"Science and technology are primary productive forces." This top-level forum was organized to mobilize the wisdom from scientists home and abroad to review the problems of viral hepatitis, liver diseases and liver cancer from a highly strategic point of view. It was hoped that by presentations, free discussion and exchange of experiences, some important directions and strategies on research of viral hepatitis and liver cancer will be made, leading to more effective control or even eradication of viral hepatitis B.

2. General overview of the forum

This forum was held on June 22, 2012 at the Shanghai International Convention Center. The date and place was chosen to coincide with the International Symposium of Viral Hepatitis and Liver Diseases, which has been a triennial symposium started in 1972. Around 1500 participants including renowned scientists and clinical experts in the field of viral hepatitis, liver diseases and liver cancer attended this symposium, which gives the forum the unique opportunity to invite a number of top experts home and abroad to discuss important issues covering different fields, as well as from highly strategic levels to provide outstanding advice directing the future of research for viral hepatitis and liver cancer and make new solutions for controlling these diseases.

This forum was organized by the Chinese Academy of Engineering (CAE), in collaboration with Fudan University Shanghai Medical College, Shanghai Consulting and Academic Activities Center of CAE and the Medical Division of CAE. The forum was presided by Prof. Daiming Fan, the Vice President of CAE, and Prof. Ji Zhou,

President of CAE, gave the opening address. Ten staffs from different departments of CAE attended the forum. Prof. Zhou commented that "top-level forums organized for development of applied sciences are sponsored by CAE to provide platforms for international exchange among top scientists to discuss important issues or problems. This forum is an excellent platform for exchanging new ideas and strategies as well as new solutions on viral hepatitis and liver cancer from integrated, strategic and perspective views. The solutions to these issues will not only result in control of diseases for human beings, but also are beneficial for economic development, social stability, and improvement of the livelihood of all peoples. Rapid developments in biotechnology are providing new opportunities and challenges for solving the problems in viral hepatitis, liver diseases and liver cancer. This forum is organized at this critical time to foresee the future directions for research and application of prevention and therapies to control these diseases." He hoped that this forum will be instructive and successful.

According to the theme of the forum, the following core topics were discussed: Future trends and new implications for prevention and treatment of viral hepatitis and liver cancer; Directions for basic and applied research; Individualized treatment; Measures for moving the focus forward to control viral hepatitis and liver cancer. These topics were first presented by speakers, including Prof. Blain Hollinger from Baylor College of Medicine, USA; Prof. Masoa Omata from Tokyo University, Japan; Prof. Michael Houghton from Alberta University, Canada; Prof. Michael P. Manns from Hanover University, Germany; Prof. Timothy Block from Drexel University Medical School, USA and Prof. Zhaoyou Tang from Fudan University Zhongshan Hospital, China; Prof. Hongyang Wang from the Second Military Medical University, East Liver Surgery Hospital, China; Prof. Jinlin Hou from Nanfang Hospital, and Dr. Yu Wang, Dr. Li Ruan from China CDC.

After the keynote presentation, active discussion was followed. Academicians from CAE, Dr. Shengli Yang, Prof. Shusen Zheng, Prof. Hui Zhuang and other experts participated in the discussion. Altogether 12 scientists from 5 countries and 14 scientists from China took parts in the forum. President Ji Zhou of CAE presented certificates for the speakers at the forum, and a group photo was taken at the end of the forum.

3. Summary of advices and suggestions

(1) Research and development of vaccines is of priority in the future for the control of

viral hepatitis and liver cancer

Though inactivated and live attenuated vaccines against hepatitis A have been widely used in China, and have shown good efficacy in prevention of viral hepatitis A, research on molecular attenuation of the virus, as well as development of vaccines based on virus-like particles and multivalent attenuated or subunit vaccines are future directions for more effective and less expensive new hepatitis A vaccines. In a recent country-wide survey, the HBsAg carrier rate in the general population in China has decreased from 9.75% to 7.18%, and among children between 1–4 years old, the HBsAg carrier rate was only 0.96%. However, it is estimated that there are 3% of infants born to HBsAg and HBeAg carrier mothers unprotected by the current hepatitis B vaccine plus hepatitis B immunoglobulin immunization. Since persistent infections are predominantly caused from infant and perinatal infections of hepatitis B virus, it is urgent to develop hepatitis B vaccines targeting these low responders and non-responders. To date, combination therapy with antiviral drugs plus interferon have shown good efficacy among hepatitis C infected patients; however, the difficulty of early identification of patients without clinical symptoms, and the cost and side effects of the treatment indicate the necessity of development of preventive vaccine against hepatitis C. Collaboration and exchange of information with some ongoing clinical trials of hepatitis C preventive vaccines outside China will be helpful for Chinese scientists. A preventive vaccine of hepatitis E virus has been developed and licensed in China. The next step is to identify the target vaccinees and detailed analysis and studies of its efficacy in mass immunization.

(2) Integrated basic and applied research on liver cancer will be of utmost importance in the coming years

Due to the effort of combination therapy, the five-year survival rate of liver cancer has increased from 7.4% to 44%. It is expected that in the coming years, liver cancer due to HBV and HCV infections will still be of immense importance for Chinese. The critical point is to discover and treat liver cancer as early as possible. Facing the multiple reported biomarkers for prediction of liver cancer, it is important to identify how the combination of certain biomarkers or a group of biomarkers can be really of use for early diagnosis and treatment of individual liver cancer patient. The use of GP73 and its fucosylated glycoform as biomarkers should be studied in different stages among different liver cancer patients and all biomarker studies should be done with appropriate

controls and proper analysis. Inflammatory microenvironment plays an essential role in the initiation and proliferation of liver cancer. Infiltration of inflammatory cells, necrosis or apoptosis and various cytokines and chemokines secreted by immune cells can remodel preneoplastic microenvironment in the liver, and can promote genetic mutations and proliferations of cancer cells and should be studied in details in liver cancer. The implications between metabolic factors, liver cirrhosis and liver cancer have also been brought up at the forum. Japanese scientist introduced their approach of using the second generation sequencing technology to do full genome analysis of liver cancer tissues, by which they predict that it will lead to clarification of the intra-individual and intra-tumoral genomic diversity of liver cancers, which, in turn, may lead to the development of new molecular targets and anti-cancer drugs. Another important viewpoint on treatment of liver cancer was presented, i. e., "Elimination plus Modification" strategy. It was also advised that research on metastasis and recurrence should be the focus for treatment of liver cancer in the future, and cancer stem cells are important targets to be investigated.

(3) Individualized treatment is the direction for viral hepatitis therapy

Currently, treatment of hepatitis B and C mainly include antiviral drugs and interferon. To further improve the efficacy and decrease the cost, individualized treatment should be considered in the future. Appropriate animal models to mimic the different stages of the disease are necessary to serve this purpose. Different therapeutic approaches and strategies should be encouraged, such as employment of various immuno-therapeutic/immuno-modulatory tools, which may either trigger innate immune responses (toll-like receptors) or specific therapeutic vaccines targeting specific genes or proteins. Gene therapy using adenovirus or other viruses as vectors, or development of reagents that can decrease HBsAg levels are to be considered. When working on these approaches, it is advised to foresee the prospects of translation to bedside, rather than only sit at the bench. In addition, it was advised that the cost of these approaches should not be neglected. Overuse of various therapies should be avoided. Since individual response will be different, methods to monitor early responsiveness to these therapies are necessary requirements prior to design individualized treatment.

(4) Establish networks for sharing resources and improving the quality of service

German scientist introduced their experience in building up the German network on viral hepatitis in collaboration with other European countries, which covered 500,000 patients

with chronic hepatitis B and 500,000 with chronic hepatitis C. The network of hep-net 2000-1012 was linked and integrated with other national webs. They described the funding sources and academic input from different universities and hospitals. Several clinical trials were successfully carried out through this web and data collected were useful for many purposes. It was advised that evidence-based and multiple center studies should be established, while studies in monosite should be discarded. Only by this means, the mechanisms of diseases, efficacies of therapeutics, reliability of diagnostic methods can be well studied and properly analyzed. In addition, the social and economical benefit and services to patients will thus be significantly improved.

(5) Reforms in medical education and medical system will influence diagnosis and therapy in viral hepatitis and liver diseases

The scientist from the US predicted that in the future two decades, there will be important reforms in medical education and medical systems which will bring new directions for hepatologists: (i) Hepatologists will co-manage patients with family doctors, physician assistants or nurse practitioners. Tool kits (simple directions for diagnosis and treatment for hepatitis patients) will be printed and distributed to them as continuous education, so patients can be early discovered, diagnosed and treated. This will save much money, otherwise those patients would be at the later stage with severe complications. (ii) Outdated methods of teaching will give way to new technology, such as online video presentations with embedded quizzes. These will help the students to learn outside the classroom, and gain practical knowledge for better service in prevention and treatment of hepatitis and liver diseases. (iii) Medical sciences will proceed hand in hand with information technology. In the future, physicians will utilize powerful digital tools, including mobile sensors, and advanced processors, which will transform our understanding of an individual. Smart phones handheld, pocket-sized visualization tool powered by ultrasound technology may be used to visually inspect the inside of a patient's body. There will be a shift to the doctor-patient relationship with the physician establishing a partnership with the patient in order to guide the patient, and hepatologists should be prepared to change.

At the forum, new ideas, new strategies and new solutions were generated at the top and strategic level, and thus the forum was a success. It was suggested that a similar forum will be organized after 3-5 years, which may check on the progress of viral hepatitis and liver cancer studies and make changes in the predictions accordingly. It is

hoped that the spirit of this forum will continue and bring genuine benefit to patients and healthy people.

Yumei Wen MD

Professor of Institute of Medical Microbiology, Fudan University, Professor of Key Laboratory of Medical Molecular Virology, Ministry of Education/Ministry of Health, Shanghai, P R China

Her interests are in research of molecular virology and immunology of hepatitis B virus, and in microbial functional genomic studies. She is one of the pioneers in basic and applied studies on therapeutic vaccine for chronic hepatitis B patients. Prof Wen graduated from Shanghai Medical University and has been a WHO fellow at London School of Hygiene and Tropical Medicine, and Fogarty visiting scholar at NIAID, National Institutes of Health, USA.

She has published 240 articles home and abroad, and has edited and co-edited 8 books, including *Viral Hepatitis in China—Problems and Control Strategies* (Vol 19, Mongraphs in Virology, Karger publisher, 1992), *Recent works on microbes and infections* (World Scientific publisher, 2009).

She was elected member of the Chinese Academy of Engineering in 1999, was member of the Editorial Board of *Archives on Virology* (2008—2010), was awarded Honorary Doctorate by University of Duisburg and Essen, Germany (2009) and Award in recognition of outstanding contribution in Medical Virology by Asian Pacific Society for Medical Microbiology. She is the co-editor-in-chief of *Emerging Microbes and Infections* (2012—).

List of Experts Attending the Forum

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Overseas Experts

Blaine Hollinger	Professor of Baylor College of Medicine, USA
Charles M. Rice	Academician of National Academy of Sciences, USA
Masao Omata	Professor of Tokyo University, Japan
Michael Houghton	Professor of University of Alberta, Canada
Michael Kew	Professor of University of Witwatersrand, South Africa
Michael P. Manns	Professor of Medical School of Hannover, Germany
Michael Roggendorf	Professor of Department of Gastroenterology, Hepatology and Endocrinology, University of Duisburg-Essen, Germany
Robert H. Purcell	Academician of National Academy of Sciences, USA
Robert Thimme	Professor of Albert-Ludwigs-University Freiburg, Germany
Steve Feinstone	U.S. Food and Drug Administration, USA
Timothy Block	Professor of Drexel University College of Medicine, USA
Howard C. Thomas	Professor of Imperial College/Honorary Professor of St Mary's Hospital, UK

Chinese Experts

Ji Zhou	President of the Chinese Academy of Engineering (CAE), Academician of the CAE
Daiming Fan	Vice President of the CAE, President of The Fourth Military Medical University, Academician of the CAE
Zhaoyou Tang	Professor of Zhongshan Hospital, Fudan University Academician of the CAE
Hongyang Wang	Professor of Eastern Hepatobiliary Surgery Institute/Hospital, Academician of the CAE
Yumei Wen	Professor of Shanghai Medical College, Fudan University, Academician of the CAE
Shengli Yang	Professor of Shanghai Institutes for Life Sciences, Chinese

	Academy of Sciences, Academician of the CAE
Shusen Zheng	Professor of the First Affiliated Hospital of College of Medicine, Zhejiang University, Academician of the CAE
Hui Zhuang	Professor of School of Basic Medical Sciences, Peking University, Academician of the CAE
Jinlin Hou	Director and Professor of the Hepatology Unit and Department of Infectious Diseases, Nanfang Hospital, Southern Medical University
Qin Ning	Professor of Tongji Hospital, Tongji Medical College of Huazhong University of Science and Technology
Lunxiu Qin	Professor of Zhongshan Hospital, Fudan University
Li Ruan	Professor of National Institute for Viral Control and Prevention, Chinese Center for Disease Control and Prevention
Zhigang Tian	Professor of School of Life Sciences, University of Science and Technology of China
Bin Wang	Professor of Shanghai Medical College, Fudan University
Jiyao Wang	Professor of Zhongshan Hospital, Fudan University
Yu Wang	Director of Chinese Center for Disease Control and Prevention
Xuanyi Wang	Professor of Institutes of Biomedical Sciences, Fudan University
Youhua Xie	Professor of Shanghai Medical College, Fudan University
Dongliang Yang	Professor of Tongji Hospital, Tongji Medical College of Huazhong University of Science and Technology
Zhenghong Yuan	Professor of Shanghai Medical College, Fudan University
Xinxin Zhang	Professor of Ruijin Hospital, Shanghai Jiaotong University School of Medicine

Part Ⅲ Keynote Speeches and Introduction to Keynote Speakers

Future Control of Liver Cancer

Masao Omata

Yamanashi Central Hospitals and University of Tokyo, Japan

Looking back at the history of hepatocellular carcinoma (HCC) treatment and the medium survival time (MST), it has been prolonged from 9 months to initial RFA (radio frequency ablation) treatment to 90 months. The local regional therapy by RFA and surgical interventions has been very successful and prolonged the patient's life (Fig. 1 and 2).

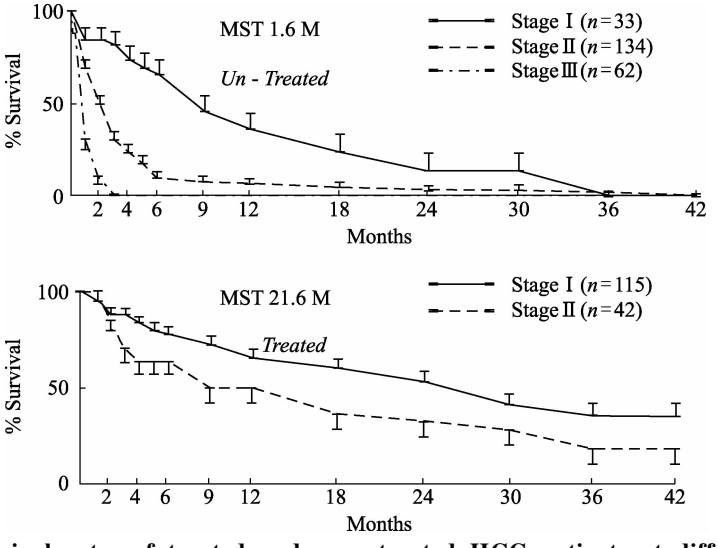

Fig. 1 Survival rates of treated and un – treated HCC patients at different stages (MST 9 M) (Okuda. Natural History of HCC;850 cases. Cancer 1985,56:918—).

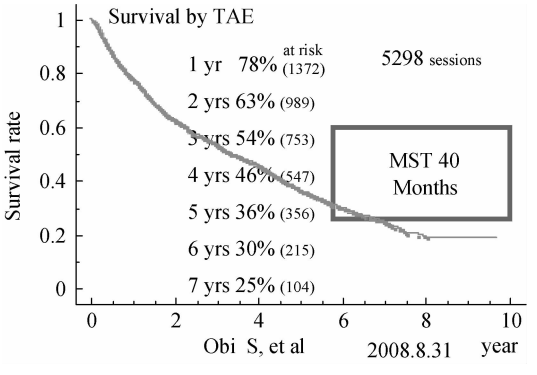

Fig. 2 History of median survival time.

HBV virus general vaccination program drastically decreases the infection and subsequently prevents the occurrence of cancer in most countries. But until then, antiviral treatment can contribute to the reductions of HCC (Hepatocellular carcinoma) infected by the viruses. Entecavir and Tenofovir are very powerful tools to suppress the HBV infections. Recent data indicates that reduction of hepatic fibrosis is the reality. Fibrosis regression by the antivirus for HBV is estimated as 0.26 metavir score per year, which is comparable to the regression rate of 0.28 reported in the HCV eradicated patient. Regression of hepatic fibrosis is certainly helpful to treatment of HCC. However, further to drastically improve the prognosis of the patients, the addition of the antiviral treatment may reduce recurrence rate from the cirrhotic background. There is certainly limitation for application of this antiviral treatment for HBV and HCV patients because interferon in particular often has side effects. The introduction of the oral preparation of the antiviral drugs for HCV infection may totally change the paradigms. Many of drugs reported so far have very slight side effects and can be used even in the patients with high Child-pugh scores. They change the paradigm of the areas in that we have the difficulty to treat HCC.

Finally, the treatment of HCC by itself has been depending on the development of the anticancer drugs; recently molecular targeting drug in particular. However, so far the overall survival benefit by Sorafenib is only 3 months (Fig.3).

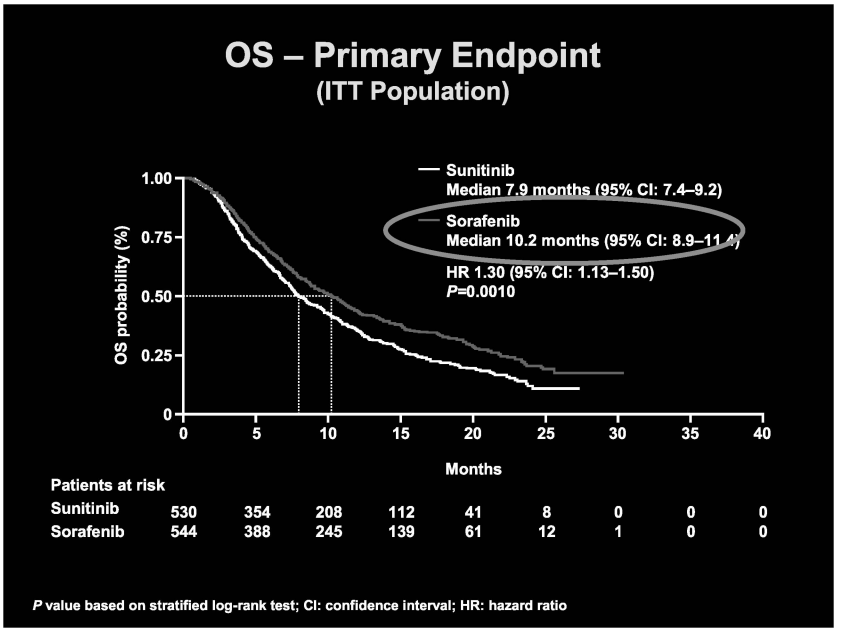

Fig. 3 Median survival time of Sorafenib.

To further advance this area, the cancer cells should be explored more by the analysis of genetic structures and expression profile (Ref). We have studied the genomics in HCC, and the application of next generation sequencing will start to clarify the intraindividual and intratumoral diversity in genomic alterations in HCC. A new sequencing era in HCC has begun (Fig. 4 and 5).

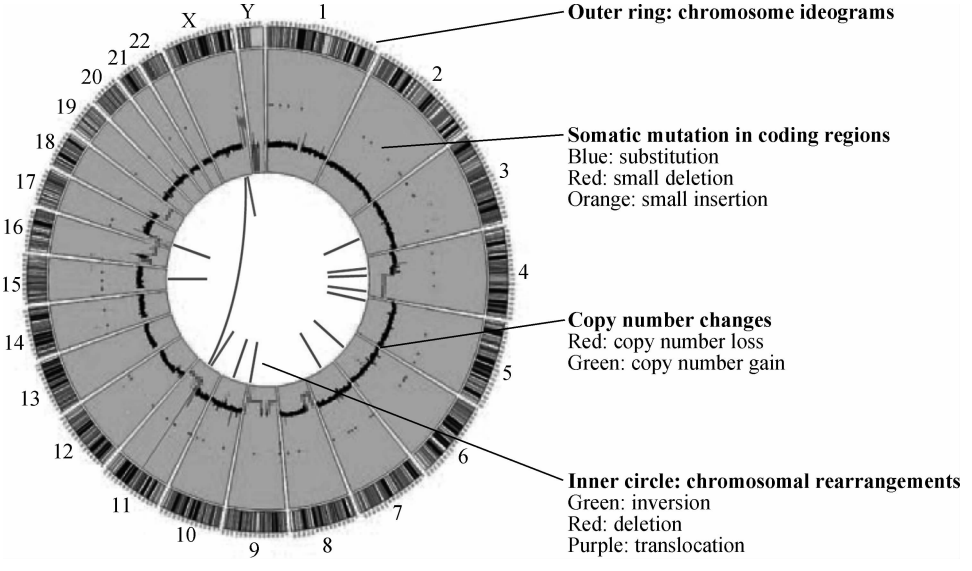

Fig. 4 Whole-genome view of somatically acquired alterations in the liver cancer genome.

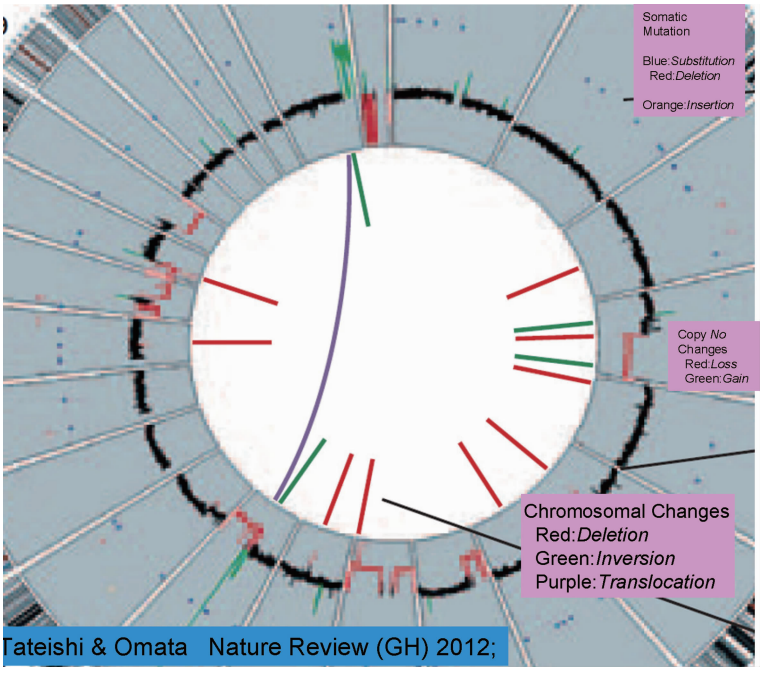

Fig. 5 Sequencing of HCC genome.

By these studies, it might be able to identify the promoter mutation which might end up in the development of the new molecular targeting drugs (Fig. 6).

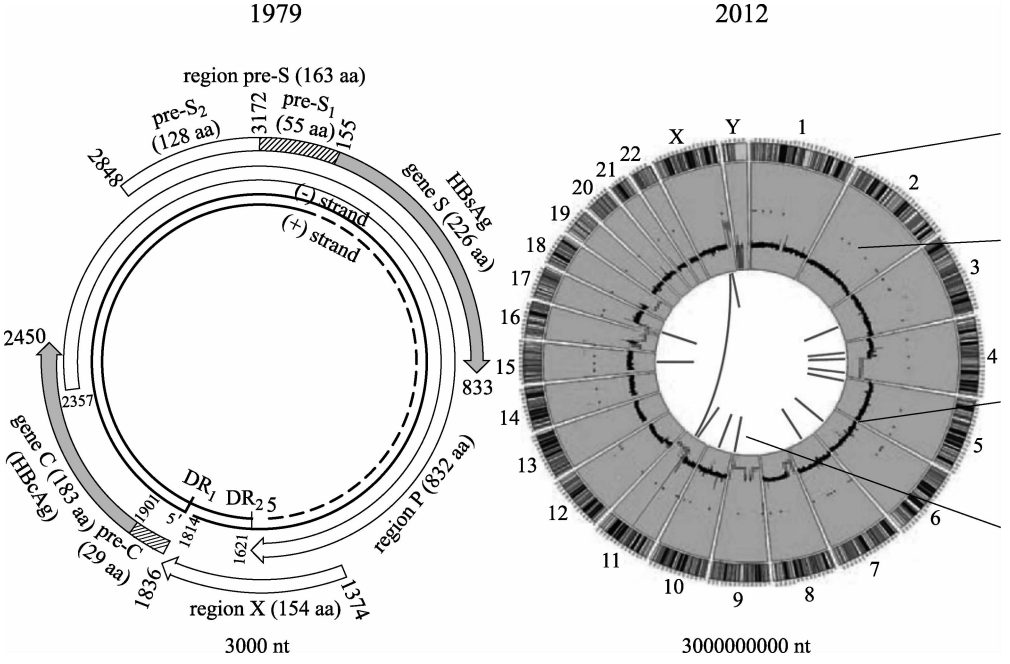

Fig. 6 The numbers of nucleotide sequences that can be sequenced in 1979 and 2012

References

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Masao Omata, M D

Department of Gastroenterology, President/Honorary professor

Yamanashi Central and Kita Hospitals/University of Tokyo

Masao Omata, MD, is a Co-Editor of *Hepatology International* (an official journal of APASL-

Asian Pacific Association for the Study of the Liver) with Prof. Sarin. He graduated from Chiba University in 1970, and joined the First Department of Internal Medicine of Chiba University chaired by Prof. Kunio Okuda. After three years' training in Japan, he went to the United States (3 years at Yale and 3 years at USC-University of Southern California, Liver Unit) where he was trained by Prof. Gerald Klatskin at Yale and Robert L. Peters at USC. The publication in *Gastroenterology* in 1979 on the etiology of hepatocellular carcinoma (one of the first papers documenting the virus etiology in USA where alcoholic liver disease was predominant) was quoted in the *Textbook of Harrison* for several editions. He was also a co-author of first documentation on "Fibrolamellar carcinoma" with Edmondson and Peters.

In 1979, he returned to Dr. Okuda's department, and started to introduce molecular biological techniques in clinical gastroenterology in 1982 and published approximately 90 papers including 2 *N Engl J Med* original articles (1985 and 1991) on molecular biological aspect of hepatitis viruses, especially on human and duck hepatitis B viruses until his move to University of Tokyo in 1992. He first reported the correlation of pre-core mutant and fulminant hepatitis, and extremely high eradication rate of acute hepatitis C by interferon, later confirmed by other groups. He first documented that the supercoiled or CCC (Covalently Closed Circular) DNA of HBV was the key molecule for the resistance of anti-viral treatment in human samples (Yokosuka, Omata, Summers, Hepatology 1985).

In 1992 (13 years after Chiba era), he was promoted to the chairman of Second Department of Internal Medicine at University of Tokyo. This was an unprecedented event in the history of Japanese medical society since the medical faculty at the University of Tokyo had never chosen the chairman of the major clinical departments from outside of University of Tokyo graduates until his appointment. Because of the re-organization of the departments in University of Tokyo, he became the chairman of Department of Gastroenterology in 1997, and has established a large and very strong department of Gastroenterology with over 100 young gastroenterologists and hepatologists.

In 2009, he was promoted to become the president of two hospitals; Yamanashi Central (691 beds) and Kita (200 beds) Hospitals to upgrade the medical care system in Yamanashi Prefecture, west of Tokyo, where, although scenic place with Mt. Fuji, hepatitis virus infection is endemic. The governor of Yamanashi has asked Prof. Omata to combat with the liver diseases of his homeland under his expertise, and also run the two hospitals as a president.

Number of publications in English peer reviewed journals in the last 19 years after he moved to University of Tokyo, is 941 in all disciplines (both basic and clinical) of Gastroenterology and Hepatology.

He was a president of the 40th Annual Meeting of Japan Society of Hepatology (10 000 members), a president of the APASL (Asian Pacific Association for the Study of the Liver) (2006 – 2007), and a president of the 50th JSGE (Japanese Society of Gastroenterology) (28 000 members) 2008 meeting.

Currently, he is an Editor-in-Chief of *Hepatology International* (the Official Journal of APASL), and is in charge of APASL-Tokyo-Secretariat.

The Rise and Fall and Rise of the GP73 and Fucosylated Liver Cancer Biomarkers

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Early detection of hepatocellular carcinoma (HCC) offers the best chance for a favorable outcome. Unfortunately, noninvasive serological markers of liver cancer have been of limited value, and the commonly used marker, alpha fetoprotein (AFP) is "positive" in fewer than 50% of the cancers. We, and others, have been using glycoproteomic approaches to discover new markers. We have reported that a resident Golgi protein, GP73 (also called "Golph 2", "GOLM1"), is elevated in the circulation of patients with a diagnosis of liver cancer. GP73 has been shown in numerous studies to have a significant ability, far superior to that of AFP, in discriminating between a diagnosis of liver cirrhosis and liver cancer. However, other studies have found that GP73 is inferior to AFP. This review describes recent advances that may explain the disparate performances of GP73 and other biomarkers in discriminating between control and disease subjects. Briefly, we have found that specific glycoforms of GP73 are far more selective for cancer than is total GP73, alone. Moreover, although GP73 is rarely elevated in people without liver disease, it is often elevated in patients with liver cirrhosis. Hence, the predictive usefulness of GP73, and other biomarkers, is enormously improved by consideration of certain clinical characteristics.

HCC is the world's 3rd leading cause of cancer death, and is responsible for nearly 1 million deaths annually[1-3]. In some regions in China, it is among the most common

causes of death amongst young men[1,2,4-6]. It is now one of the few cancers increasing in incidence in the US[1,2,4-7].

Outcome is greatly improved by early detection[8,9]. Serum proteins that can help in risk stratification or can become part of early detection procedures could be of enormous significance, bringing patients to diagnostic imaging and cancer management when tumors are small and treatable. The current, noninvasive marker, AFP and its fucosylated glycoform, L3, and even the newer markers, such as DCP, are of limited value, detecting cancer in roughly, only between 30%-70% in all cases[10]. Of course, AFP and L3 are of no value in detecting AFP negative HCC, which accounts for between 25%-50% of all HCC[11].

We have discovered and reported that a normally resident Golgi membrane protein, called GP73, is found in the circulation of patients with a diagnosis of liver cirrhosis and HCC, and serum levels of the protein may be useful in HCC detection[12,13]. There are now several independent reports that generally confirm our results regarding GP73 as a possible biomarker of HCC[14-18] or of liver disease[19-22].

Most remarkably, a recent "blinded" study of more than 4000 people, ~800 of whom had HCC and 600 had cirrhosis, showed that GP73 was much more sensitive and specific for HCC than AFP[22].

Indeed, although GP73 is elevated in kidney cancer[23] and prostate cancer derived seminal fluid[19,24], its secretion into the blood appears to be very liver cancer selective[25]. However, there are also reports which, although are consistent with findings that GP73 is associated with HCC and liver disease, do not conclude that its detection in the serum offers any better performance than AFP[20,26]. Along these lines, several studies from our laboratory have provided examples where GP73 and the fucosylated acute phase proteins perform better, and others in which they perform less well, than does AFP in discriminating between cirrhosis and liver cancer samples, and this is summarized in Table 1.

If and how these promising markers should be used is a significant question. Since some studies show they are accurate, should they be used? Since some studies show they are inferior to AFP, should they be dropped?

Table 1 Summary of biomarker performance in distinguishing between liver cirrhosis and HCC from our studies

		Marker[1]				
	Controls/HCV/HBV/ Cirr/HCC (n)[2]	AFP[3]	GP73[3]	f-hem[3]	f-kin[3] & f – AIAT	Reference[4]
Study 1	23/0/23/0/8	N/A	0.98[5]	N/A	N/A	12
Study 2	56/0/0/152/144	0.61	0.79	N/A	N/A	13
Study 3	20/0/20/20/20	N/A	0.80	0.95		27
Study 4	00/00/00/113/164	0.83	0.89	N/A	0.79	24
Study 5	0/0/0/200/100	0.80	0.64	0.62	0.70/ 0.55	Ref set/with IFN failure samples, unpublished
Study 6	20/133/33/32/72	0.82	0.90[5]/ 0.89	0.95[5]/ 0.87	0.80	25

[1] Marker utilized in study. AFP' Alpha-feto protein; GP73, Golgi Protein 73; f-hem, fucosylated hemopexin; f-kin, fucosylated kininogen. Assays as in grant text and citations.

[2] Number of individuals in the given study with the following clinical characteristics: Controls, no evidence of liver disease; HCV, infection with HCV but no evidence of liver cirrhosis or HCC; HBV, HBV infection but no evidence of liver cirrhosis or HCC; Cirr (cirrhosis); HCC, hepatocellular carcinoma. Diagnosis and distribution of viral etiologies comprising HCC and cirrhosis as in grant text or citation.

[3] For each marker the AUROC at differentiating HCC from cirrhosis is given, excepting superscript [5]; kininogen (kin), Alpha 1 antitrypsin (A1AT).

[4] Citation of publication of study.

[5] Ref sets including with rebetron failures.

[5] AUROC for HCC against all other disease categories in study.

This review will consider the physical properties of GP73 and compare the clinical characteristics of people in a study in which GP73 performed well, as a biomarker of HCC, and a study in which it did poorly.

GP73 is elevated in people with a diagnosis of liver cirrhosis

Study after study has confirmed that GP73 levels in the blood are higher in patients with a diagnosis of liver cancer, as compared to healthy subjects and those with

hepatitis[10,13-18,20,22]. However, as we observed in our initial report of GP73 as a biomarker of disease, it is often elevated in patients with liver cirrhosis, and no evidence of HCC[12,19,28]. Consistent with these reports, as shown in the scattergram (Fig. 1) that combines data from age and gender matched samples from studies 4 and 5 (Table 1) from our group, GP73 levels tend, as a general rule, to be highest in patients with HCC, individuals with liver cirrhosis are indeed more likely than those with hepatitis and no cirrhosis, to have high levels of GP73, although these levels are usually less than that in liver cancer. This, suggests, that although GP73 levels, alone, may indicate a pathological situation, they must be combined with other information to be reliable in making a risk assessment of HCC.

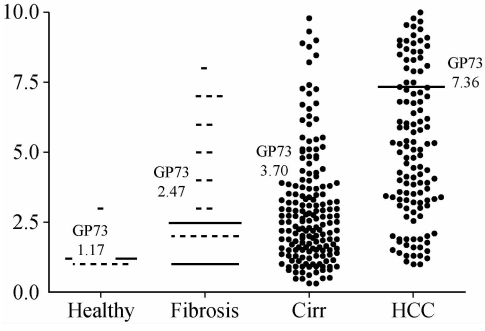

Fig. 1 GP73 is elevated in liver cirrhosis as well as HCC.

The serum levels of GP73 were determined as in[24], by an ELISA method. The data is based on samples from two studies of ~250 individuals with liver cirrhosis and no HCC and 150 with cirrhosis and HCC, of which approximately 100 with cirrhosis and 50 with HCC had been treated with interferon alpha and ribavirin (manuscript in preparation). Levels of GP73 in people as a function of liver disease status. Mean value for each group indicated in red, relative to reference control healthy subject serum.

Glycoforms and specific fucose linkages correlate with HCC

Our observation of a correlation between serum levels of GP73 and a diagnosis of HCC was originally based on identifying GP73 as a fucosylated (f) glycoform associated with HCC[12]. Briefly, we and others, discovered that fucosylated N-glycans, derived from all proteins in the blood, are significantly elevated in patients with HCCs[12,29-31]. We then identified sets of specific fucosylated glycoproteins that were elevated in the serum of patients with HCC, compared to those with cirrhosis, and called the set the HCC/liver

cancer "fucome."[25,31]

The leading candidate proteins are GP73 and its fucosylated glycoform (although distinguishing fucosylated from un-fucosylated glycoforms forms is not routinely done). We believe these proteins are produced and over-secreted into the blood by, or because of, the cancer cells. Assays that do not distinguish between these glycoforms may lead to under performance of the biomarkers.

Thus, our work suggests that the glycoforms of the biomarkers may vary the most with disease status, and explain false positivity (cases where GP73 and f-acute phase proteins are elevated in the absence of HCC). For example, serum levels of f-GP73 (f-GP73) and a number of f-acute phase glycoproteins (i.e. f-kin, f-A1AT) are much better biomarkers of HCC than are total levels of those glycoproteins[24].

However, fucose is attached to the N-glycan at different sites and with different linkages. Following a detailed analysis of the glycoforms of A1AT, we have recently found (Fig.2) that it is the a1,6 "core" fucosylated glycoform of f-A1AT that is most HCC selective, and only one of the isoforms is specifically core fucosylated[32].

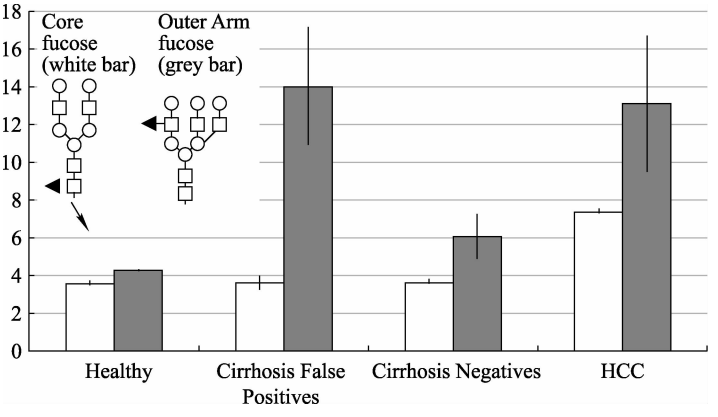

Fig.2 Levels of core, not outer arm, fucosylated A1AT correlate with a diagnosis of HCC.

Inset: N linked glycans with dark triangle illustrating either core (structure on the left) or outer arm (structure on the right). Bars indicate relative amounts of either core (white) or outer arm (grey) fucosylated A1AT levels in the circulation of age and gender matched healthy, cirrhotic individuals who had high levels of total f-A1AT (were false positive for HCC), or low levels of total f-A1AT or had a diagnosis of HCC. See[33].

Briefly, the amounts of core F(1,6)A2G2 and outer arm A3F(1G3) fucose structures from A1AT from 20 HCC and 20 cirrhosis pts who were "false positives" (had elevated total f-A1AT compared to healthy subjects) was determined by this method with scatter plots reported in[34]. Cirrhosis derived A1AT had elevated outer arm, but not core structures. Both core and outer arm was elevated in HCC A1AT. Thus, core was

only elevated in the HCC A1AT.

The reasons that core f-glycoforms appear to selectively rise in the blood of people with HCC, as compared to those with cirrhosis and no HCC, is under investigation (data not shown). It has been hypothesized that core fucose is a mechanism whereby liver glycoproteins are sorted to the bile in polarized hepatocytes[35]. We have hypothesized that, since cancer cells "depolarize," and tight junctions that establish hepatocytes' polarity become damaged in the diseased liver, core fucosylated glycoproteins become mis-sorted and are misdirected to the blood. Regardless of the reason, we speculate that knowledge of cancer specific glycoforms of the biomarkers and the ability to detect those specific glycoforms, such as core fucosylated A1AT, will result in significantly improved cancer detection (Block et al, in preparation).

Use of patient demographics and clinical information in interpreting a biomarker levels' significance

In addition to, and probably related to, a biomarker's glycan profile, we believe that incorporation of the patient's clinical profile associated with each sample can be useful in improving the performance of a biomarker.

Unfortunately, biomarker discovery and development is usually carried out with specimens far removed from the many demographic and clinical factors with which it is associated. However, these factors can be extremely relevant and useful in placing a biomarker's level into context. For example, consider the differing clinical features associated with the populations in two studies. In one study, GP73 performed well in distinguishing between cirrhosis and HCC, and in the other, GP73 did poorly (Table 1, studies 4 and 5, respectively). There are a number of factors that stand out and highlight differences between the mean clinical values of those in the controls (cirrhosis, no HCC) and those in the cases (cirrhosis, HCC).

First, compared to the patients in the study where GP73 performed poorly, the "controls" (those with no detectable HCC) in the study in which the biomarkers distinguished well between the cases and controls, age was lower, they were interferon treatment naïve, and otherwise presented with a much better compensated liver disease profile (lower baseline ALTs, ASTs, Alk phosphatase). The affect of clinical factors upon GP73 levels is summarized in Table 2.

Table 2 Clinical factors affecting GP73 levels[1]

	Affect upon serum GP73
Increased age	Increases
Increased ALK PHOS[2]	Increases
Increased MELD[3]	Increases
IFN failure[4]	Increases
Liver cirrhosis	Increases

[1] The affects of the patient information shown in the first column upon serum GP73 levels is indicated in the second column. This is based upon our analysis of data from studies 4 and 5 Table 1). The indicated patient variable, alone, was associated with increases in serum GP73 even in the absence of HCC.

[2] ALK PHOS is serum alkaline phosphatase, which may be elevated as a result of bile elements in the circulation.

[3] MELD, Model for end stage liver disease.

[4] IFN, interferon. This is based on HCV patients who have been treated within and failed to reach efficacious clinical milestones, with IFN and ribavirin.

Since these differences might reflect or be a function of the different clinical compositions of the populations, the possibility that including clinical factors along with the GP73 and f-hem values could improve biomarker performance in the population in which the biomarkers on their own did so poorly. Therefore, looking at the age and gender matched samples from the poor performing groups in studies 4 and 5 (Table 1), the ability of combinations of values of the biomarkers was used with logistic regression analysis using GP73, f-hem and clinical variables, to determine which, if any, clinical variables contributed to discrimination between a diagnosis of cirrhosis and HCC. The results suggested that, if age and gender are accounted for, significantly improved discriminators can be achieved. For example, in distinguishing between liver cancer and liver cirrhosis, using GP73 and f-hem values, with age and gender, an AUROC of 0.75 was achieved. Thus, although this is significantly less than the 0.81 value achieved using AFP alone, it is much better than the 0.65 value of using GP73 alone (Study 5, Table 1).

Taking these factors together, regression analysis was used to construct a decision tree, which provides instruction as to how to interpret GP73 levels, in the context of clinical information. Fig. 3 shows a "Decision Tree" to illustrate how GP73 levels, considered with other clinical factors, can be used to assign a patient's risk of liver cancer. The risk factor value of GP73 levels for liver cancer becomes extremely impressive.

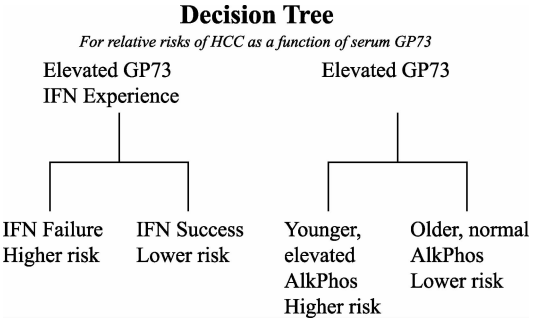

Fig. 3 Decision Tree for use of GP73 values in assigning an individual's risk of HCC.

Individuals are sub-grouped as a function of IFN treatment, and then by age and alkaline phosphatase. Specific odds ratios and risk rates are currently being calculated and will be reported elsewhere (Evan et al, and Devarajan et al, in preparation).

Conclusions

Despite previous reports, some from our group, in which elevated GP73 distinguished between HCC and cirrhosis, the present study alerts us to the reality that GP73, alone, may not be sufficient to achieve this discrimination in every population. This is because many other clinical factors may be influencing the levels of this biomarker. This review attempts to identify some of these clinical variables, and help provide a means of using these clinical values, in combination with GP73, to achieve the best use of the entire clinical biomarker family.

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Education and Career Background

PhD: State University of New York

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Biography

Dr. Timothy Block is the Co-Founder and volunteer President of the Hepatitis B Foundation, which is a national non-profit organization dedicated to finding a cure and improving the lives of those affected worldwide through research, education and patient advocacy. Dr. Block is also Professor of Microbiology and Immunology, Drexel University College of Medicine, and Director of the Drexel Institute for Biotechnology and Virology Research. Dr. Block has been involved in research for more than 25 years. Honors include a Lifetime Achievement Award from the Centrals Bucks Chamber of Commerce for his humanitarian efforts in addressing the global health problem of hepatitis B, honorary Medical Doctor and election into the Bulgarian Academy of Medicine, Distinguished Service Recognition from the National Cancer Institute's Early Detection Research Network and a Special Citation from the US House of Representatives in recognition of "outstanding achievements." Dr. Block is also an elected Fellow, International Union Against Cancer and an elected Fellow, Glycobiology Institute of The University of Oxford. In 2009, Dr. Block was named an elected Fellow of the American Association for the Advancement of Science (AAAS). Most recently, Dr. Block was named judges' choice CEO of the Year in the Philadelphia Business Journal's Inaugural Life Sciences Awards (2010), and named one of 100 Most Inspiring People in the Life-Sciences Industry in 2011 by PharmaVoice Magazine.

Research Interests

Dr. Block's lab is studying chronic viral diseases. The molecular mechanisms of herpes simplex virus latency continue to be an important area. However, most of the work is in the area of hepatitis B and C and liver cancer. With a glycobiology research theme, his lab searches for antiviral agents and their mechanism of action (two of the discoveries have led to therapies now in human clinical trials); for biomarkers of disease, using glycoproteomics (two of the discoveries have led to biomarkers being tested in people for the early detection of liver and colorectal disease); and to understand disease by examining the role of protein folding in antigen presentation and how the viruses antagonize and oppose the host innate immune system.

Perspectives of Clinical Study of Hepatocellular Carcinoma

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The last half-century witnessed the exciting progress of clinical study of hepatocellular carcinoma (HCC). Based on the pathology, several kinds of therapeutic methods for eradication of HCC have been developed and have improved survival rates of pat ients. Data collected in the Department of Surgery, Liver Cancer Institute, Fudan University, showed that 5-year survival rate was increased every decade (7.4% in 1968–1974), validating the achievements earned in clinical treatment and research of liver cancer. However, statistical analysis of the whole population of HCC (e.g. in the last three decades in USA, 5-year survival rates were 4% in 1975–1977, 6% in 1884–1986, and 14% 1999–2005 (Jernal et al. *CA-Cancer J Clin* 2010), showing that though the 5-year survival rate was improved there still is a huge gap to achieve conquering HCC. By looking ahead in the future, clinical studies of liver cancer still confront with several challenges especially in the several fields as below.

Early diagnosis and early therapy are still of importance and serve as the major strategy to improve the therapeutic efficacy of liver cancer

As I mentioned above, improvement of 10-year survival rate has been ascribed to early diagnosis and early therapy, because the mean diameter of cancer tissues at each stage of liver cancer is 11 cm, 9 cm, 7 cam, and 5 cm respectively. Altekruse et al (Altekruse et al. *Hepatology* 2012) reported that 5-year survival rate of cases of liver cancer in 1975–1977 was 3% and was increased to 18% in 1998–2007, which was considered to benefit from early diagnosis and early therapy of more patients. Therefore, it could be inferred that survival rate could be further improved by

continuously strengthening the survey of high-risk population. Recently, some new markers, such as Golgi protein 73, for early diagnosis of liver cancer were reported, but their accuracy was showed comparable to alpha fetoprotein (AFP) (Zhou et al. *BMC-Cancer* 2012). Shang et al reported that osteopontin was more sensitive than AFP for early diagnosis of liver cancer (Shang et al. *Hepatology* 2012). Tomimaru et al claimed that miRNA-21 in plasma is better than AFP for the early diagnosis (Tomimaru et al. *J Hepatol* 2012), but its application in surveillance of high-risk population is yet to be investigated. Giannini et al still insisted on the use of AFP for surveillance of liver cancer (Giannini et al. *Hepatology* 2011). In my opinion, currently, periodical surveillance of high-risk population by AFP combined with ultrasonic imaging still serve as the practical methods. The ratio of early-stage patients, who were received in our institute and diagnosed during physical examination, increased. However, the positive diagnosis rate of AFP only accounts for 50%-60% of liver cancer patients, so it remains a critical issue to search for new and easy-to-manipulate markers for early diagnosis. Taken together, early diagnosis and early therapy is an efficient way and perspective with long-term significance to improve the therapeutic efficacy of liver cancer.

Combined treatment is the long-term strategy

Integration of "eradication + modulation" warrants attention. Achievement of various therapeutic methods to eradicate cancers, especially for the early-stage patients has been well recognized. Five-year survival rate of HCC patients with small tumor in our institute was: 57.9% for patients with resection (totally 5767 cases), twice that for HCC patients with larger tumor (31.5%, 5345 cases); survival rate was 47.9% for patients with radio-frequency ablation (482 cases); 76.9% for the patients with liver transplantation according to Milan Criteria (193 cases). However, since HCC could not be completely resolved, how to control the residual HCC after these radical treatments remains to be investigated. Cancer is a complicated disease, which is developed through multiple stages, involving multiple genes and processes, and keeps on changing, so it is impossible to find specific effective drugs, similar to that as for treating bacterial infections. Preliminary results showed that the therapeutic efficacy of molecular targeting against one or several limited number of molecules is far less than expectation. Therefore, combined treatment is a long-term strategy. The combined treatment employed in the latter part of last century was "eradication + eradication" (e.

g. surgery and radio/chemotherapy), which was mainly based on the idea that cancer is a local lesion. This strategy is still being used and remains to be further explored. For example, it was reported that the efficacy of radio-frequency ablation (RFA) combined with transcatheter arterial chemoembolization (TACE) to treat small HCC was better than that of single use of RFA (Morimoto. *Cancer* 2010), but the outcome was not as good as expected. In the future, investigations focusing on modulation of residual cancer, by modulating the micro-environment, and responses of the patients will be indispensable. Combined application of these investigations and therapeutic methods for eradicating HCC represents an important direction. This can be achieved not only by focusing on the local tumor, but also taking the whole body of patients into account.

Recently, a number of combined therapeutic approaches have emerged, including eradication methods combined with modulation: (1) Combined with cellular factors. Post-resection or post-RFA followed by administration of α-interferon improved the therapeutic efficacy to certain extent. It was firstly discovered by our institute that combined with α-IFN, the recurrence of HCC metastasis was decreased by inhibition of angiogenesis (Wang et al. *Hepatology* 2000). The therapeutic efficacy was confirmed by randomized-controlled clinical study. Meta-analysis of radical treatments of 1180 HCC cases showed that 2-year survival rate without recurrence was improved to 35.4% by combined treatment with IFN (Shen et al. *J Hepatol* 2010). It was reported that 3-year survival rate of late-stage HCC patients by combination therapy with intra-arterial 5-fluorouracil and pegulated IFN reached 44% (Kasai et al. *Cancer* 2011). (2) Combined application of anti-inflammatory agents. Inflammatory micro-environment promotes tumorigenesis and is regarded as the 7th characteristic of tumor (Mantovani. *Nature* 2009). Inflammatory responses play decisive roles at different stages of tumor development, including initiation, promotion, invasion, and metastasis. Inflammation also affects immune surveillance and responses to therapies (Grivennikov et al. *Cell* 2010). A number of anti-inflammatory agents are available for treatment or are under development, including aspirin and other nonsteroid anti-inflammatories, anticytokine therapies and small molecules that block the activity of kinases, and small RNAs (Dinarello. *Cell* 2010). Our study found that combination of zoledronic acid (ZA) depleting macrophages by inhibiting tumor angiogenesis and metastasis improved the antitumor effect of sorafenib (Zhang et al. *Clin Cancer Res* 2010). (3) Combined application of molecular targeted therapy. Sorafenib was approved for use in advanced

HCC patients. Sorafenib suppressed the development of postsurgical intrahepatic recurrence and metastasis by using an orthotopic xenograft model of HCC (Feng et al. *Hepatology* 2011). The recent trend is combined use of Sorafenib and other molecular targeting drugs or chemotherapy. For example, efficacy of treatment with sorafenib plus doxorubicin was better than that of doxorubicin monotherapy (Abou-Alfa et al. *JAMA* 2010). (4) Combined systemic intervention. Tumor is a systemic disease. Chronic stress altering immunological, neurochemical and endocrinological functions enhanced tumor angiogenesis and promoted malignant cell growth (Thaker et al. *Nat Med* 2006). Therefore, systemic intervention deserves attention and could be fulfilled via treating nervous, immune, endocrine, and metabolic systems. Although it is still immature in the field of systemic intervention, the perspective of its development is promising, especially in controlling residual tumor by combination therapy after tumors were removed. Combined immunotherapy warrants special attention. It was reported that ASPH-loaded DCs reduced HCC recurrence by activating antigen specific $CD4^+$ T cells (Shimoda et al. *J Hepatol* 2012). In some patients treated with adoptive immunotherapy using dendritic cells pulsed with tumor lysate, decrease of AFP and release of γ-IFN were observed (Palmer et al. *Hepatology* 2009). Two most recent reports showed that androgen receptor (AR) inhibited HCC metastasis (Ma et al. *Hepatology* 2012) and thyroid hormone receptor suppressed HCC metastasis efficiently, which suggested that intervention of endocrine system could be possible. Pegylated arginine deiminase (ADI-PEG 20) can keep the late stage of advanced HCC at a stable status (Yang et al. *Br J Cancer* 2010), which provides a clue for the efficacy of intervention of metabolism in HCC patients. (5) Combination therapy of traditional Chinese medicine (TCM). The efficacy of TCM to eradicate tumors may be low, but TCM could be possible to modulate tumor cells and achieve survival with tumor in HCC patients. In fact, combined prescription of TCM frequently used for treatment of HCC showing comprehensive effects including anti-inflammation, improvement of microcirculation, anti-angiogenesis, and improvement of immune responses, etc. TCM treatment emphasizes to prescribe on individualized basis, which may possibly help personalized therapy based on a systemic level. Our study found that Chinese herbal extract Songyou Yin (composed of five herbs) downregulated expression of MMP-2 and VEGF, induced apoptosis, suppressed tumor growth to some degree, and prolonged the survival time of mice with tumor. In addition, Songyou Yin upregulated E-cadherin, inhibited tumor metastasis

induced by oxaliplatin treatment, and extended mouse survival (Xiong et al. *BMC-Cancer* 2010). (6) Other combined therapy with potential efficacy. Recently, a panel of new therapeutic targets and clues such as microRNAs, molecules associated with HCC (especially the molecules related with signaling pathway and HCC stem cells), molecular regulation of immune and endocrine systems, and normalization of endothelial cells, anti-mitosis pathways have been presented. For example, rifampicin for treatment of tuberculosis could be used as an oral angiogenesis inhibitor targeting hepatic cancers (Shichiri. *Cancer Res* 2009). Among all these, microRNAs deserve attention. miR-139 inhibited HCC metastasis by downregulating Rho-kinase 2 (Wong. *Gastroenterology* 2011). miR-7 also inhibited HCC metastasis by interfering with the p13K/AKT pathway (Fang. *Hepatology* 2012). Whereas, miR-135 was found to promote HCC metastasis (Liu. *J Hepatol* 2012). Some of the miRNAs seemed to show the therapeutic potentials. Furthermore, some therapeutic methods for eradicating HCC promoted metastasis of residual cancer. Our study suggested that eradication of HCC had certain negative effects on promoting metastasis of residual cancer. Palliative resection of HCC upregulated expression of matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF) and promoted metastasis of residual HCC (Li. *Cancer Gene Ther* 2011). Chemotherapy by oxaliplatin downregulated expression of E-cadherin and promoted metastasis (Liu. *Clin Cancer Res* 2010). Nexavar also promoted invasion and spread of residual HCC. Similarly, some recent publications showed that the spread of cancer was actually accelerated by choking off a tumors' blood supply (Hayden. *Nature* 2009). Anti-angiogenesis inhibitors reduced primary tumor growth but promoted tumor invasiveness and metastasis (Loges. *Cancer Cell* 2009).

Therefore, efficacies of frequently used therapeutic methods to eradicate tumors should be acknowledged, but negative effects of these methods also need to be noticed and studied. The current therapeutic methods are "shortcuts" to treat, which need to be improved. Our preliminary results showed that combined administration of some routine used drugs or biologics, such as IFNs, TCM compound prescription of "Songyou Yin," tyroserleutide, zoledronic acid, aspirin, oxymatrine, and tanshinone could alleviate these negative effects.

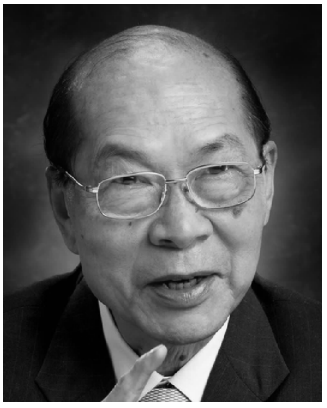
The bottleneck of HCC metastasis and recurrence

The data we have and other reports suggested that 5-year survival rate of HCC (60%–

80% for liver transplantation, 50%–60% for small HCC resection, 30%–40% for big HCC resection, 30%–40% for RFA of small HCC, 20%–30% for TACE) by all therapeutic methods seemed to have reached the top limit. The bottlenecks are mainly metastasis and recurrence. In our institute, therapeutic efficacy of resection of small HCC in last 4 decades has not been improved; the 5-year survival rates of each decade were 57.9%, 57.9%, 55.5%, and 58.1%, respectively. These data showed that there have been no new methods to solve the issues of metastasis and recurrence.

Fortunately, in the field of HCC metastasis, some new knowledge recently emerged: (1) HCC metastasis is not a phenomenon of advanced period. One study showed that metastasis progression was initiated in the primary tumors and even small HCC could have a high potential of metastasis (Ye. *Nat Med* 2003). (2) HCC metastasis is closely correlated with immune-inflammation micro-environment. A refined 17 genes of noncancerous hepatic tissues could be used for prediction of HCC metastasis (Budhu. *Cancer Cell* 2006). Hence, study on HCC metastasis should not only focus on cancer cells, while it is hopeful to understand how to modulate the "soil (micro-environment)." (3) Metastasis potential of HCC is a double-edged sword (either to be better or to be worse). Eradication methods of HCC mentioned above either can promote HCC metastasis or compromise the potentials of metastasis. (4) Cancer stem cells are critical for HCC metastasis. Interactions between cancer stem cells and matrix cells in the micro-environment promote the formation of metastasis niche (Malanchi. *Nature* 2012). (5) Gene profiles during metastasis of cancer cells are kinetically changed (Clifford. *Nature* 2012). These information will provide clues for designing novel therapeutic methods to combat HCC metastasis.

Taken together, it is a long-term task to do clinical study of HCC. The core task is still to extinguish tumor; however, modulation of the tumor and the body as a whole is an indispensible supplement. Studies on liver and tumor biology are key factors to further improve the survival life span of HCC patients.



Prof. Zhaoyou Tang

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Professor Zhaoyou Tang is an internationally renowned liver cancer researcher and the founder for early diagnosis and treatment of hepatocellular carcinoma. He is also one of the first elected academicians of Chinese Academy of Engineering, Division of Medicine and Health, honorary fellow of American Surgical Association, and honorary member of Japan Surgical Society. He is currently the chairman, professor of surgical oncology, and PhD supervisor of Liver Cancer Institute, Zhongshan Hospital, Fudan University. He had been council member of International Union against Cancer (UICC), Hepatoma Professional Committee Chairman of the Chinese Anti-cancer Association, vice president of the Chinese Medical Association, and president of Shanghai Medical University.

He first systematically proposed the concept of "subclinical hepatocellular carcinoma" and is the chief editor of "*Subclinical Hepatocellular Carcinoma*" (in English). Hans Popper, the founder of modern hepatology, praised this study by "this concept represents major progress in the understanding, and particularly the management, of hepatocellular carcinoma." The study of small liver cancer doubled the 5-year survival rate of the patients underwent curative surgery, and promoted the transformation of hepatocellular carcinoma treatment from "incurable disease" to "part-can-be-cured disease." In recent years, he further carried out "the metastasis and recurrence research of hepatocellular carcinoma." Then he established the first human liver cancer nude mice model with high metastastic potential and cell lines with different metastatic potentials and targets and successfully used it to study the metastasis of hepatocellular carcinoma. He had been twice the Liver Cancer Conference chairman of the International Cancer Congress, more than 90 times been the invited speaker at international conferences, 7 times as the chairman hosted Shanghai International Symposium on Liver Cancer and Hepatitis. He is also the editorial board member of 11 international journals and the editor-in-chief of two journals in Asia-Pacific region. He edited 9 monographs and participated in editing other 16 international monographs. He published 288 articles, which were cited for 6759 times; in liver cancer area, this achievement

ranked No. 3 worldwide (No. 1 on the mainland, China). He was honored with a gold medal of "Early Treatment and Early Cure" from USA in 1979. In addition, he was awarded first prize of the National Science and Technology Progress Award twice, the third prize twice, Ho Leung Ho Lee Science and Technology Progress Award, Chinese Medical Science Award, China Engineering Science and Technology Award, Wu Jieping Medical Prize, Bethune Medal, "May Day" Labor Medals, and Shanghai Science and Technology Hero. Until now, 59 PhD students under his supervision have graduated, and four of them got National Excellent Doctoral Dissertation Award.

Nonresolving Inflammation and Metabolic Abnormalities Promote Hepatocarcinogenesis

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Liver cancer ranks the fifth most common cancer worldwide and the third leading cause of cancer death. Its poor prognosis mainly attributes to the lack of curative methods and effective diagnosis at early stages. And the mechanism of liver cancer has not yet been fully understood.

Among the many etiologies of hepatocellular carcinoma (HCC), hepatitis accounts for over 75% of all HCC occurrences. During the long preneoplastic progress following hepatitis, the liver undergoes fibrosis and cirrhosis and dysplastic hepatocytes were gradually produced, giving rise to HCC. Although the genetic and/or epigenetic basis of the malignant phenotype of HCC is heterogeneous, it is clear that the chronic inflammation in the liver is closely associated with the malignant transformation of hepatocytes. Numerous studies suggest a close association between nonresolving inflammation in the liver and the occurrence of HCC, and it is acknowledged that an inflammatory microenvironment plays an essential role in the initiation and proliferation of HCC. The infiltration of inflammatory cells may be brought out by liver cancer cells, or by hepatocytes undergoing necrosis or apoptosis. Various cytokines and chemokines secreted by immune cells as well as direct contact between surface proteins of immune cells and liver cancer cells remodel preneoplastic microenvironment in the liver, in the favor of genetic mutations and proliferations of cancer cells. Tumor necrosis factors (TNFs) and interleukins (ILs) are the most studied signal molecules in the microenvironment of HCC. These cytokines activate transcription factors, such as NF-

κB, STAT3 and AP－1, which, in turn, mediate tumorigenesis, metastasis and angiogenesis via regulating a variety of downstream target genes. The rate of HCC decreases after the cause of nonresolving inflammation is abolished.

Cancer arises from a rare group of cells, termed cancer initiating cells (CICs) or cancer stem cells (CSCs). These cells occupy a distinct subpopulation within the tumor and are responsible for both initiation and progression of the cancer. Increasing evidence of heterogeneous pathologies and hierarchical organizations of HCC suggests the existence of liver CSCs which sustains the growth and metastatic potential of HCC. CSCs possess at least three distinct characteristics: the ability to self-renew, differentiation capacity and tumorigenicity. Adult hepatocytes maintain stem cell-like properties because they are capable of extensive proliferation upon liver damage as well as differentiate into biliary lineages under special conditions. Thus hepatocytes may transform into liver CSCs through dedifferentiation under proper microenvironment and initiate HCC. Another possible cellular origin of liver CSCs is dysregulated liver stem/progenitor cells. Our most current study also demonstrates that HBx could promote the expansion of hepatic progenitor cells and give rise to CSCs in liver.

Recently, the relationship between metabolic factors and chronic liver diseases including liver cirrhosis (LC) and HCC has become a hot topic, such as diabetes, obesity, alcohol consumption, and intake of aflatoxin-contaminated food.

Metabolic syndrome (MS) has been recognized as a major public health problem worldwide arousing more attentions. MS is a collection of metabolic abnormalities, including abdominal obesity, blood lipid barrier, diabetes, and hypertension. MS is interrelated with insulin resistance, which is also known as insulin resistance syndrome and associated with a chronic proinflammatory state. Notably, obesity and diabetes are also likely to be risk factors for HCC. In a recent systematic review of 13 case control studies, 11 reports supported an association between diabetes and the development of HCC. Of the 13 case control studies, subjects with diabetes had a 2-fold higher risk of HCC. The chief pathway by which obesity increases risk involves the association between obesity and nonalcoholic fatty liver disease (NAFLD). NAFLD is the most common cause of chronic liver disease among adults in western countries. NAFLD comprises a spectrum of conditions, ranging from fat alone to fat plus inflammation, fat plus ballooning degeneration, and nonalcoholic steatohepatitis (NASH), which is a well-recognized cause of cirrhosis and has been increasingly associated with the

development of carcinoma.

As the hepatic entity of metabolic syndrome, NAFLD/NASH is a risk factor for HCC, even in the absence of cirrhosis. A recent study has shown that NAFLD is a principal risk factor in the development of HCC, irrespective of age. Accumulating evidences also suggest that visceral adipose tissue secretes vascular endothelial growth factor (VEGF) and other adipokines, implicating the dysregulation of angiogenesis as a connection between obesity and worse clinical outcome. In animal models, leptin also activates many signal transduction pathways, such as JNK, protein kinase B, AKT, and the extracellular signal-regulated kinase pathway in HCC cells, all of which promote the progression of cancer.

Alcohol consumption increases the risk of HCC primarily through the development of cirrhosis, and coffee consumption appears to have a favorable effect on the prevention of liver diseases, including HCC. There are several hypotheses to explain the reasons.

HCC is a complicated disease involving various aspects of both host and environment. Since hepatitis virus infection is directly linked to HCC onset, development and application of potent anti-virus agents is essential in the prevention of HCC. Genetic and histological heterogeneity renders the poorly therapeutic effect of current strategy. Therefore, molecular classification of HCC was anticipated to favor the personalized management of HCC in future. Comprehensive analysis regarding the distinct role of each factor as well as the cross-talking network among them is necessary to improve the curative effect of HCC. Combinations of new target-specific drugs and chemotherapy would pivotally enhance the anti-tumor efficacy. The use of 'cocktails' of target-directed drugs that aim at different hallmark characteristics of HCC are likely to achieve maximal therapeutic benefits. Although recent progress on HCC study offers promise to translate bench-top discovery to clinical practice and shed new light on the prognosis of HCC patients, further studies are in urgent need to further improve the diagnosis and therapy of HCC.

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Prof. Dr. Hongyang Wang received her doctor's degree from University Ulm, Germany in 1992. She set down and became professor and director of International Cooperation Laboratory on Signal Transduction in 1997, and chief physician and director of Department of Clinical Treatment Ⅱ in 1999 in Eastern Hepatobiliary Surgery Institute and Hospital in Shanghai. And in 2005, she was elected as academician of Chinese Academy of Engineering. In 2009, she was elected to be the first director of the Medical Department of National Natural Science Foundation of China (NSFC).

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New Individualized Treatment in Chronic Hepatitis B Patients

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Chronic hepatitis B (CHB) infection is a significant health problem throughout the world, and particularly in China. It is estimated that more than half a million Chinese people die annually from end-stage hepatitis B complications, which is associated with huge healthcare costs and a heavy socioeconomic burden. The goal of therapy for CHB is to improve quality of life and survival by preventing progression of the disease to cirrhosis, end-stage liver disease, HCC and death. There are two kinds of approved anti-hepatitis B virus agents including interferons (standard and two pegylated interferons) and nucleos(t)ide analogues (NAs) (lamivudine, adefovir dipivoxil, entecavir, telbivudine and tenofovir).

One major effort should be made to improve the antiviral efficacy and to reduce the risk of viral resistance due to poor response to the current available antiviral strategy, depending on exploring the valuable predictors of responsiveness. The points of optimized antiviral strategy are to initiate treatment with a proper drug at the proper time point, to monitor the on-treatment response closely and, in the patients with poor response, to consider adding on a second drug or switching to another more potent drug. Optimization of antiviral strategy for CHB patients includes either interferon based response guided therapy (RGT) approach or NAs based treatment (either start with high potency and low resistance NAs, or start with high resistance (LAM/LDT)/low potency (ADV) NAs, and then modify strategy according to patients' response (Roadmap Concept).

Interferon based response guided therapy (RGT) approach

One study in China is aimed to identify how treatment of HBeAg-positive patients with

peginterferon α-2a can be optimized using a response guided therapy. At the time of analysis, 261 patients had completed 24 weeks of PEG-IFN α-2a therapy. Overall, 25% (66/261) had met the criteria of early response (HBV DNA <5 Log copies/mL and HBsAg <1500 IU/mL after 24-week peginterferon treatment). Of the early responders, 26% (17/66) had achieved HBeAg seroconversion at week 24 versus 6% (11/195) in non-early responders. Baseline HBsAg/HBeAg quantitation and HBV DNA were significantly lowered in early responders than those in non-early responders. Changes from baseline in HBsAg, HBV DNA and HBeAg were more pronounced in early responders (−1.48 log IU/mL, −4.78 log copies/mL, −1.57 PEIU, respectively) than those in non-early responders (−0.41 log IU/mL, −2.47 log copies/mL, −0.82 PEIU, respectively). This study will provide valuable information on how patients with CHB can benefit from baseline and response guided therapy to help optimize treatment duration and the final results are eagerly anticipated.

NUCS start in naïve patients

Entecavir and Tenofovir, characterized by high potency with low resistance, have been recommended as the first line (AASLD 2010 and EASL 2012) or preferred (Chinese guidelines 2010 and APASL 2012) regimens for naïve patients by major guidelines. However, due to the high cost of the first line agents, there are only 13% naïve patients using entecavir as the first antiviral strategy, while, over 70% patients are using LAM/ADV/LDT, which bring large cohorts of patients with suboptimal response or/and viral resistance.

NUCS based roadmap/salvage strategy

Patients starting with high resistance drugs such as LAM/LDT should be closely monitored, and doctors should modify the treatment according to patients' response by adding on no-cross resistance NAs in case of drug resistance. About 2/3 patients will require adding another drugs according to optimizing therapy strategy; adequate lab support and good patient compliance are key success factors. The one key RCT study (EFFORT study) is ongoing in China, patients receiving optimized treatment achieved significantly higher rate of HBV DNA <300 copies/mL (71.7% [215/300] vs. 58.5% [175/299], $P=0.001$), lower rates of virological breakthrough (1.7% [5/300] vs. 12.0% [36/299], $P<0.001$) and genotypic resistance (1.3% [4/300] and 10.7%

[32/299], $P < 0.001$) than those receiving standard of care treatment. W24 add-on ROADMAP concept was proved effective in this randomized controlled study, in which W76 significantly increased antiviral efficacy and reduced viral breakthrough rate versus standard of care.

Future perspectives (EASL APASL Guideline)

In the future, more important data, focusing on optimization of the efficacy of antiviral agents, will be released from China, based on the launched National Project of Hepatitis Research. Both economic development and healthcare system reform, including a new reimbursement policy, will make antiviral agents more accessible to Chinese patients. Ultimately, it will allow for physicians greater opportunities to follow international and Chinese treatment recommendations.

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Perspectives for Better Treatment of Chronic Hepatitis B

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World Health Organization estimates that about 2 billion people worldwide have been infected with hepatitis B virus (HBV). Since the introduction of preventive vaccination programs against hepatitis B in over 170 countries, the number of new infections is continuously decreasing. However, despite the success of prophylactic vaccines, chronic HBV infection is still a global health problem. Over 360 million people are persistently infected with HBV, of whom 1 million die each year from HBV associated liver cirrhosis or hepatocellular carcinoma (HCC). Due to unstable political and economical situation in several countries the vaccination program are not as effective as it could be. The outcome of HBV infection varies greatly from person to person. In most of the cases the infection is cleared spontaneously; however, 5%–10% of adults develop chronic infection. By contrast, 40%–90% of children who are born to HBV infected mothers will develop a persistent liver disease[1].

In recent years a marked progress has been made in the treatment of chronic hepatitis B. Currently, the two types of antiviral therapies are approved: treatment with pegylated interferon alpha 2a (PEG-IFNα) or nucleos(t)ide analogues, such as adefovir, entecavir, lamivudine, telbivudine and tenofovir[2-5]. However, the efficacy of those therapies in preventing liver cirrhosis and HCC is still limited. Treatment with PEG-IFNα leads to a sustained antiviral response in only one third of patients, regardless of combining the therapy with polymerase inhibitors. On the other hand, the treatment with nucleos(t)ide analogues significantly suppresses HBV replication that leads to a decrease of necroinflammation in the liver. However, those antivirals cannot completely

eradicate the virus. After withdrawal of the drug, the rebound of viremia is observed in the majority of patients. Furthermore, the long-term treatment is subsequently associated with the appearance of drug-resistant HBV strains that is often the cause of the therapy failure[6,7]. Therefore, the new approaches in treating chronic hepatitis B are urgently needed.

Immunological control of HBV infection

It is well documented that an appropriate adaptive immune response is required to efficiently control the HBV infection. T cell-mediated immune response directed against hepatitis B virus antigens is crucial for resolution of the infection[8-12]. HBV-specific $CD8^+$ T cells are able to clear HBV-infected hepatocytes by secretion of Th1 antiviral cytokines, such as interferons (IFNs) and tumor necrosis factor alpha (TNFα), and direct cytotoxic mechanisms (perforin/granzyme, ligand-ligand induced cell death, e.g., Fas-Fas-L)[12-16] which eliminate infected hepatocytes (Fig. 1). An early, vigorous, polyclonal and multi-specific cellular immune response against the viral proteins is associated with the clearance of hepatitis B in acutely-infected patients. In contrast, chronic HBV carriers demonstrate weak, transient or often undetectable $CD8^+$ T cell response that correlates with HBV persistence[17-21]. Humoral immune responses, especially neutralizing anti-envelope antibodies, play a key role in preventing HBV spread to non-infected hepatocytes[20, 22].

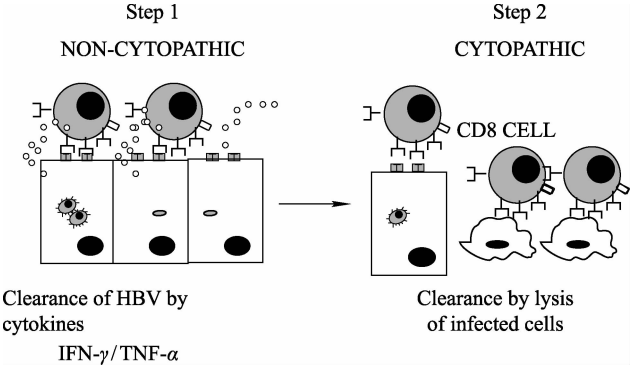

Fig. 1 Control of HBV infection by CTL inhibition of HBV replication by cytokines (IFN-α and IFN-γ) elimination of infected cells by cytotoxic activity of HBV specific CD8 cells[Desigse, A. Bertoletti].

Recent studies indicate that several mechanisms may be involved in the loss of the function of HBV-specific T cells during chronic hepatitis B. It was shown that high-level viremia negatively influences the virus-specific immune responses. High viral replication

in the liver with viral load higher than 10^7 copies/mL is correlating with hyporesponsiveness of virus-specific $CD8^+$ T-cells in patients with chronic hepatitis B[23]. Moreover, the prolonged exposure to viral antigens occurring during the chronic viral infections can trigger the T cells to become tolerant and prone to apoptosis (Fig. 2). The interaction between programmed death 1 (PD-1) receptor and its ligand PD-L1 (also known as B7-H1) plays an important role to prevent an overreaction of the immune system[24]. Recent studies revealed that inhibitory molecules such as PD-1 and CTLA-4 are markedly up-regulated on virus-specific T cells, resulting in exhaustion of those cells (e.g., lack of IFNγ production and proliferation)[25]. Simultaneously, this mechanism can contribute to the development of the chronic infection by impairment of the effective anti-viral response. This hypothesis was previously proven for hepatitis C virus (HCV)[26,27] and human immunodeficiency virus (HIV) infection in humans[28-30], as well as lymphocytic choriomeningitis virus (LCMV) infection in mice[31,32] and more recently for HBV[33,34]. Furthermore, several studies imply that functional defects of antigen presenting cells (APCs), mainly dendritic cells (DCs), may contribute to the impaired T cell response in chronic hepatitis B patients[35-41]. *In vitro* studies showed that DCs isolated from HBV chronic carriers produce lower amount of antiviral cytokines, such as type I interferons and TNFα, in comparison to healthy controls[35, 36]. In addition, those DCs are less efficient in T cell activation and stimulation of T cell proliferation[35,39-41]. The novel report demonstrated that myeloid DCs from chronic HBV patients express increased level of inhibitory PD-L1 molecule and therefore may down regulate functions of HBV-specific T cells[39].

Several investigations underline the significance of $CD4^+$ $CD25^+$ regulatory T cells in pathogenesis of persistent viral infections[42]. In HCV and HIV-infected patients it was shown that regulatory T cells may down-regulate HCV- and HIV-specific $CD8^+$ and therefore influence the disease progression[43-45]. The role of regulatory T cells in HBV infection is still not clear. Nevertheless, the increased numbers of $CD4^+$ $CD25^+$ regulatory T cells were detected in the blood and the liver of patients with chronic severe hepatitis B[46]. Recent results in mice have shown that Tregs down regulate antiviral effects of T cells through limiting cytokine production[47]. In addition, the liver itself is an organ with tolerogenic properties that might contribute to the immunological tolerance during chronic HBV infection[48,49]. Finally, viruses developed the strategies to efficiently evade the host immune response resulting in persistent infections. Viral immune escape

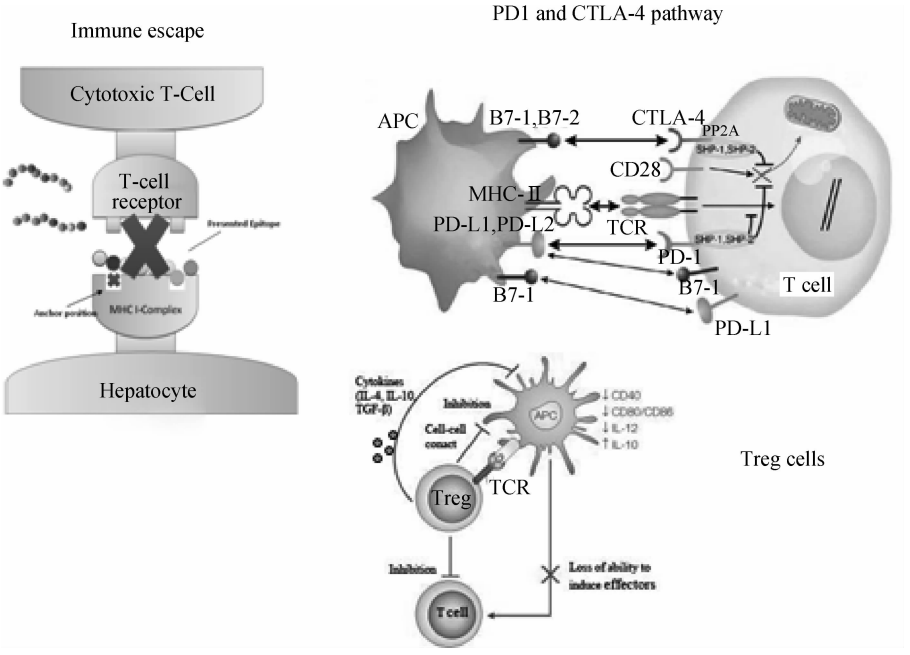

Fig. 2 Mechanisms to evade immune defense: Dysfunction of CD8$^+$ cells (Nature Immunol 3, 2201 – 2205)

due to the mutation of CD4$^+$, CD8$^+$ and B cell epitopes in a given HLA background has been observed in patients infected with HIV, HCV and HBV[50-55].

Several studies demonstrate that the treatment with lamivudine alone, or in combination with interleukin-12 (IL-12) result in the restoration of the HBV-specific CD4$^+$ and CD8$^+$ immune response in chronic HBV-infected individuals. However, the therapeutic effect was not sustained in those patients[56-58].

In this article we would like to describe new therapeutic strategies to treat chronic hepatitis B which may be introduced for patient treatment in the future. We will describe (1) new therapeutic immunization approaches, (2) HBV specific small interfering RNA (siRNA), (3) TLR as stimulators of innate immune response, (4) genetherapeutic approaches, (5) new antiviral molecules inhibiting HBsAg secretion, (6) immunomodulatory strategies. In addition to PEG-IFN-α or nucleos(t)ide analogues the following therapies may be evaluated in patients and implemented in the therapy.

1. Therapeutic immunization in chronic hepatitis B

Over the last 20 years, continuous efforts have been undertaken to develop a therapeutic vaccine for chronic hepatitis B to enhance the virus-specific immune responses and overcome persistent HBV infection[59-72].

Numerous clinical trials of therapeutic immunization exploited the conventional prophylactic hepatitis B surface antigen (HBsAg)-based protein vaccines. These studies demonstrated reductions in viremia, HBeAg/anti-HBe seroconversion and HBV-specific T cell responses in some patients. However, the antiviral effect was only transient and did not lead to an effective control of the HBV[59-66]. Combination of the HBsAg protein vaccines with antiviral treatment with lamivudine did not lead to a satisfactory improvement of the therapies[67-69].

The strategies designed to specifically stimulate HBV-specific T cell responses were also not successful[70-72]. The lipopeptide-based vaccine containing a single cytotoxic T lymphocyte (CTL) epitope derived from HBV nucleocapsid was able to induce a vigorous primary HBV-specific T cell response in naïve subjects[73]. However, in HBV chronic carriers the vaccine initiated only poor CTL activity and had no effect on viremia or HBeAg/anti-HBe seroconversion[70]. The DNA vaccine expressing small and middle envelope proteins proved to elicit the HBV-specific cellular immune response in chronic HBV carriers; however, this effect was only transient[71].

Yang et al. presented the novel DNA vaccine for treatment of chronic hepatitis and combined the immunizations with lamivudine treatment[72]. The multigene vaccine contains five different plasmids encoding most of HBV antigens and human IL-12 gene as a genetic adjuvant. The combination therapy led to sustained antiviral response in 6 out of 12 HBV chronically-infected patients. The responders were able to clear HBeAg and had undetectable viral load at the end of 52-week follow-up. Those effects were correlating with a detectable T cell response to at least one of the HBV antigens[71]. Nevertheless, further studies are needed to evaluate this strategy on a larger cohort of HBV chronic carriers.

The therapeutic vaccine based HBsAg complexed with human anti-HBs was proposed by the group of Wen et al.[74]. Immunogenic complexes (IC) stimulate robust T cell responses by increasing uptake of HBsAg through Fc receptors on APCs and, therefore, modulate HBsAg processing and presentation. It was demonstrated that this vaccine administered to HBeAg-positive patients led to decrease of HBV DNA in serum, HBeAg seroconversion and development of anti-HBs in part of the subjects[75]. Currently, the IC-based vaccine is the only one that entered phase Ⅲ of clinical trials in chronic hepatitis B patients[76]. Even though the IC-based vaccine led to antiviral effect, clearance of HBV was observed in about 15% of treated patients. It seems that the

vaccine alone is not sufficient to achieve the full control over HBV. The ongoing clinical trial will show, whether IC are effective as a therapeutic vaccine in chronic hepatitis B.

A recent study in France using nucleos(t)ide analogues treatment and therapeutic DNA vaccine to stimulate a CD4/CD8 T cell response to HBsAg was conducted. In this double blind study 32 patients who became HBV DNA negative under nucleos(t)ide analogues therapy receive five DNA vaccinations expressing HBsAg. However, the relapse rate after stopping antiviral therapy in the vaccine group did not differ from the patients who received only antiviral therapy [Pol S., Michel M., personal communication]. The failure of this treatment may be explained by the fact that nucleos(t)ide analogues reduce virus replication; however, have little influence on this secretion of HBsAg which has a major tolerizing effect in chronic hepatitis B. T cell response to the core protein or the polymerase protein may be more effective.

Animal model for studies on therapeutic immunization in chronic hepatitis B

Over the years, various animal models, including chimpanzees, woodchucks, ducks, and HBV transgenic mice, were established for development and evaluation of novel therapeutic vaccines. Studies using HBV transgenic mouse models demonstrated that DNA immunization with the expression plasmids encoding different HBV proteins could induce HBV-specific antibodies and stimulate CTL responses. However, the functionality of HBV-specific CTLs induced in transgenic mice may be not fully developed[77-79]. Improvement of DNA vaccination regimen[80] and blockade of PD – 1/PD – L1 interaction[34,81] (see below) could enhance functional T cell responses and lead to inhibition of viral replication *in vivo* without causing hepatitis. Thus, the animal models with naturally occurring hepadnaviral infection are required for the long-term evaluation of the therapeutic effect. In comparison to chimpanzees, woodchucks are easily available and affordable.

In the woodchucks, the application of DNA vaccine expressing woodchuck hepatitis virus (WHV) core antigen (WHcAg) in combination with antivirals led to the prolonged control of viral replication[82,83]. However, it became clear that the usage of the more potent vaccines is required to overcome WHV persistence. New DNA plasmid (pCGWHc) and recombinant adenoviruses (AdVs) showing high expression levels of WHcAg were developed. Mice vaccinated with the improved plasmid pCGWHc elicited a stronger WHcAg-specific $CD8^+$ T-cell response compared to the previously used

vaccines[Kosinska A. et al., under review]. The prime-boost regimen examined in this study induced a robust and functional WHcAg-specific T-cell response in mice. The vaccination with the new plasmid DNA vaccine and recombinant adenoviral vectors elicited a significant CTL response in naïve woodchucks and led to the rapid development of anti-WHs antibodies and resolution of WHV infection after WHV challenge. This WHcAg-based DNA prime-AdV boost immunization was also used in chronically WHV-infected woodchucks in combination with a potent nucleot(s)ide analogues treatment[Kosinska A. et al, unpublished results]. A significant WHcAg-specific and WHsAg-specific T cell response in all woodchucks that received the vaccination and antiviral treatment but not in control animals could be observed. Moreover, two out of four woodchucks from combination therapy group remained WHV DNA negative from the end of antiviral treatment up to the end of the monitoring period and developed anti-WHs antibodies. These promising data indicate that combination therapy using antiviral drugs which reduce viral load and a potent T cell vaccine against the HBV core protein may be a therapy for chronic hepatitis B in the future.

2. HBV specific small interfering RNA (siRNA)

RNA interference (RNAi) using small interfering RNAs (siRNAs) of a length of 21 to 23 nucleotides represents a promising way for the specific treatment of HBV infection[84]. It is mediated by chemically synthesized or vector-expressed siRNAs, which lead to the sequence-specific degradation of target messenger RNAs (mRNAs). Many clinically relevant viruses including the HIV, HBV, and HCV could be inhibited *in vitro*[85-87]. Some siRNAs have been shown to inhibit HBV gene expression and replication in transient and stable transfection systems. We were able to demonstrate that two siRNAs targeting the S and X regions of WHV genome had the highest efficacy to deplete 70% of WHV transcripts and replicative intermediates in primary woodchuck hepatocytes[88]. In addition, the expression of cellular genes involved in the innate and specific immune responses like MxA and MHC I was upregulated after siRNA-mediated suppression of WHV. Thus, siRNAs might be useful as novel antiviral agents for the treatment of chronic HBV infection, both by the inhibition of HBV replication and enhancement of the expression of cellular genes relevant for antiviral actions. However, the efficient delivery of siRNA *in vivo* has to be optimized for successful treatment.

3. TLR as stimulator of the innate immune response in chronic hepatitis B

The innate immune system represents the first line of defense against viral infections and responds at the first encounter with the pathogens. It induces an antiviral state in infected cells and activates immune cells by producing cytokines and chemokines, thereby limiting viral replication and coordinating adaptive immunity. The mammalian cells are equipped with an array of germ-line encoded pathogen recognition receptors, in particular Toll-like receptors (TLRs) that are able to recognize a set of evolutionary highly conserved structures, so called pathogen-associated molecular patterns. Based on the ability of TLR ligands to stimulate immune and somatic cells, *in vitro* and *in vivo* testing of TLR ligands was performed to examine the direct antiviral and indirect immunomodulatory effects of drug candidates. TLR ligands showed anti-HBV activity in various systems[89]. The induction of both IFN-dependent and independent antiviral mechanisms are involved in this activity. The recent findings imply that Toll-like receptor 2 (TLR2) plays an important role in the pathogenesis of chronic HBV infection[90,91]. In patients and woodchucks, relatively low levels of TLR2 expression were found on PBMCs and in liver tissues from chronically infected carriers. In the woodchuck model, TLR2 expression on PBMCs was inversely correlated with WHV DNA titers in acute WHV infection and in entecavir treated chronic WHV carriers. It could be shown that TLR2 ligands activated NF-κB, PI3K/Akt, and different arms of MAPK signaling pathways and induced the production of pro-inflammatory cytokines in hepatocytes. TLR2-mediated innate immune responses led to reduction of HBV/woodchuck hepatitis virus (WHV) replication and gene expression in human hepatoma cells and primary woodchuck hepatocytes. These data suggest that the mutual inhibition of HBV replication and TLR2 signaling represents an important aspect of HBV infection.

In addition, many TLR ligands are also known as excellent vaccine adjuvants and therefore tested for prophylactic and therapeutic vaccine formulations for HBV.

4. Genetherapeutic approaches

Alpha interferon (IFN-α) and IFN-γ play an important role in resolution of acute hepatitis B. IFN-α and IFN-γ are able to suppress Hepadnavirus replication. The intrahepatic expression of high levels of IFN may enhance the antiviral activity. In chronically HBV-infected patients, the antiviral effect of IFN-α has been demonstrated;

however, suppression of replication is observed in 37%, loss of HBeAg is induced in 33%, and loss of HBsAg is seen in 8% of patients[92]. In addition, a large number of chronically HBV-infected patients are excluded from IFN-α treatment, e.g., patients with normal alanine aminotransferase levels do not profit from this therapy, and patients with advanced cirrhosis cannot be treated because of severe side effects[93]. The treatment is accompanied by side effects, e.g., flu-like symptoms in most patients, weight loss, bone marrow suppression, an increased risk of sepsis (particularly in those patients with cirrhosis), alopecia, thyroid dysfunction, and depression and other psychiatric disorders[94]. The local expression and action of IFNs may be a new therapeutic approach to reduce side effects and enhance the antiviral effects. High-capacity or helper-dependent Ad (HD-Ad) vectors seem to be good candidates for the delivery of cytokine genes to the liver. HD-Ad vectors do not express any viral proteins, and thus safety is considerably improved; in addition, the immune response induced by the vector itself is significantly reduced compared to previous-generation Ad vectors[95]. Gene delivery with HD-Ad vectors was also shown to induce a long-lasting expression of proteins. The effects of wIFN-α and wIFN-γ on chronic hepadnavirus infection in the woodchuck has been tested by a new approach: we wanted to achieve local and long-lasting expression of IFN-α or IFN-γ in the liver *in vivo* by delivery of the respective cytokine genes to hepatocytes via HD-Ad vectors. The transduction of livers of WHV carriers *in vivo* with HD-AdwIFN-α or HD-AdwIFN-γ induced levels of biologically active IFN, which could be measured in the sera of these animals. Expression of wIFN-α in the liver reduced intrahepatic WHV replication and WHV DNA in sera of about 1 log step in two of two woodchucks[96]. Alternative vectors or hepatotropic recombinant viruses, e.g., hepatitis A virus or hepatitis D virus, may be used to deliver the expression of IFN-α and -γ. However, so far no recombinant and attenuated hepatitis A virus or hepatitis D virus has been reported.

5. Inhibition of release of HBsAg from infected hepatocytes

Infected hepatocytes release large quantities of complete viral dane particles and small 22 nm particles containing only envelop protein. By nucleotide treatment viral replication is inhibited; however, the release of small 22 nm particles is not inhibited. These HBsAg particles, which have been associated with HBV-specific immune defects and may contribute to the long term persistence of HBV infection in the liver may be responsible

for continuation of tolerance. Inhibition of the release of HBsAg particles may be a strategy to gain function of T and B cell response. Recently preliminary results of a new compound have been presented which specifically inhibit release of HBsAg in DHBV infected ducks [Gilbert A., unpublished results] and in patients (press release REPLICOR).

REP 9AC' represents the latest clinical candidate in a new class of antiviral agent that blocks the release of hepatitis B surface antigen (HBsAg). Pre-clinical studies in ducks with DHBV infections showed a significant drop of DHBsAg during treatment with REP 9'AC. The clinical candidate, REP 9AC, rapidly cleared serum HBsAg also in patients infected with HBV. This is exemplified by the first patient who had 2,000,000 copies of the virus/mL in his blood, was then treated for a total of 23 weeks and who has so far maintained a sustained virologic response (<70 copies of the virus/mL in his blood) for a period of 6 months off treatment. This reduction of HBsAg by combining REPLICOR with nucleos(t)ide analogs may be a new option to reduce viral load and secretion of HBsAg and allow many of these patients to regain effective immunological control over their infection, resulting in sustained virologic responses. REP 9AC may therefore provide an important new tool in the treatment of chronic hepatitis B.

6. Immunomodulatory strategies

As mentioned above the down-regulation of CD8 T cell function by the interaction of PD-1 and PD-L1 may attribute to chronic outcome of acute hepatitis B[24, 33, 34, 39]. Blocking this interaction by specific antibodies may lead to gain of functions. Pre-clinical studies in the woodchuck model have shown that T cell function can be restored. Characterization of wPD-1 and wPD-L1 of woodchucks revealed a high similarity to the counterpart of mammalian species[97]. Expression of wPD-1 on CD8 T cells during acute infection correlated with viremia and CD8 T cell response[Liu J. and Roggendorf M., unpublished results]. wPD-1 was elevated in PBMCs of woodchucks with chronic WHV infection. *In vitro* blockage of wPD-1/wPD-L1 interaction by antibodies enhanced lymphoproliferation and CD107a degranulation in woodchucks with acute or chronic WHV infection. Woodchucks chronically infected with WHV were treated with potent antiviral drug entecavir and received repeated DNA vaccines (WHV core and WHsAg). During the vaccination, antibodies against wPD-L1 was intravenously applied. These antibodies strongly restored T cell function in entecavir treated and DNA vaccinated

woodchucks. These results indicated that wPD－1/wPD－L pathway may play an important role in T cell tolerance in chronic WHV infection. *In vivo* blockage of wPD－1/PD－L pathway could strongly enhance T cell functions and break immunological tolerance to viral antigens in chronic WHV carriers.

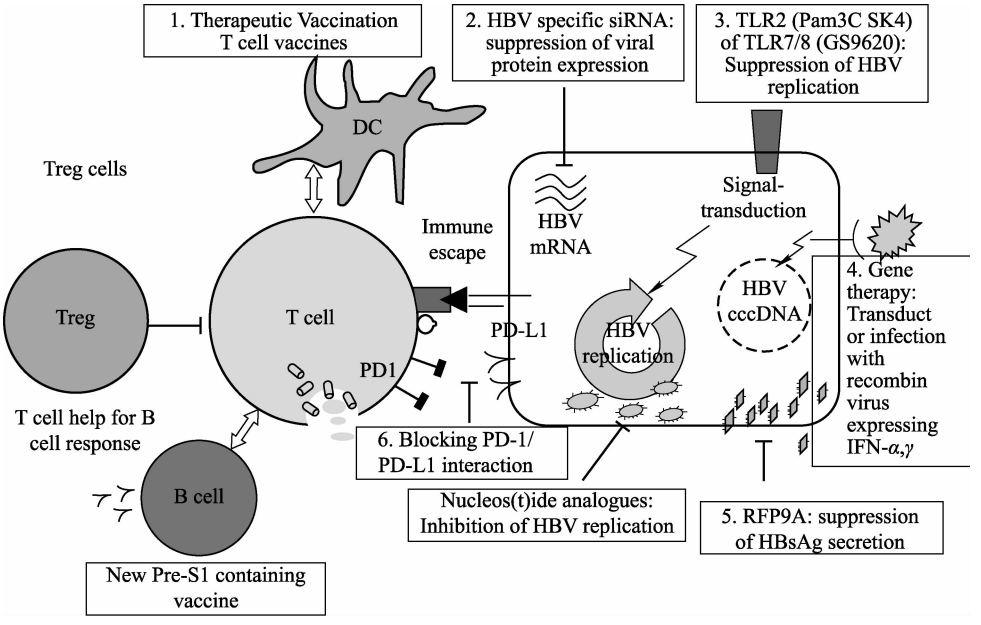

Fig. 3 New approaches for the treatment of chronic hepatitis B most probably combination of different procedures may be necessary for elimination of HBV.

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His scientific key aspects are the pathogenesis and prevention of viral hepatitis and retroviral infections.

He is the head of the National Reference Center for Hepatitis C and the National Reference Laboratory for Rabies, member of the Advisory Board of the Deutsche Leberstiftung and Chairman of the Diagnostic Committee of the German Society for Virology.

Clinical Studies and New Therapies—The Role of Networking

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The pharmaceutical industry has created its own networking to perform phase 1—3 trials which are necessary for drug development to bring drugs to approval and markets. However, investigator initiated and sponsored trials (IIT, IST) are necessary to develop new therapies for orphan diseases and orphan indications and to optimize therapies with already approved drugs.

The German network of competence "Hep-Net" (www. kompetenznetz-hepatitis. de) was founded in 2002. After a 12. 5 million public funding by the German Government (BMBF) over the first 8 years, Hep-Net is now a project carried on forward and funded by the German Liver Foundation (www. german-liverfoundation. com; www. deutsche-leberstiftung. de). One of the projects of Hep-Net is the **Hep-Net Study House** which performs clinical studies mainly as investigator initiated trials (IIT) but also as investigator sponsored (IST) trials with support by the government, the German Liver Foundation and the pharmaceutical industry. Among these trials are the German Hep-Net Acute Hepatitis C Studies. We were able to create a network of over 70 centers contributing to these trials that could establish the benefits of early treatment with interferon monotherapy to prevent chronicity.

Recently, the Hep Net Ⅲ study was completed comparing immediate interferon monotherapy with the wait and see strategy in a prospective manner. Furthermore, we performed multicenter trials in order to individualize PEG-IFN plus ribavirin therapies and to establish optimized duration of treatment for hepatitis C based on early viral kinetics. The results of some of these studies entered national as well as international practice

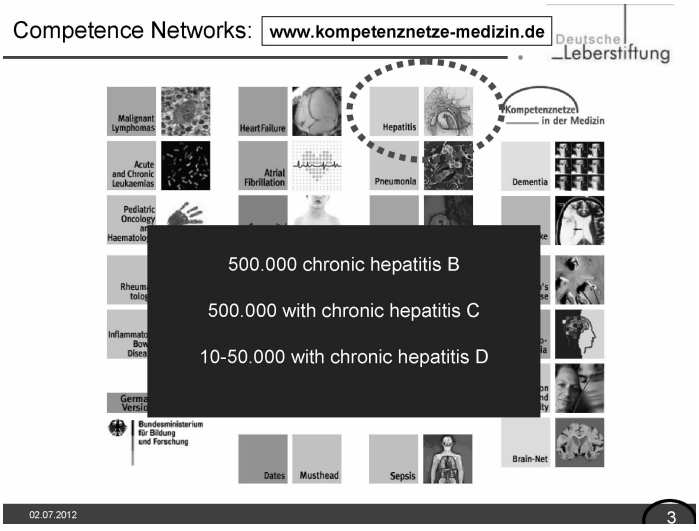


Fig. 1 The competent network in Germany

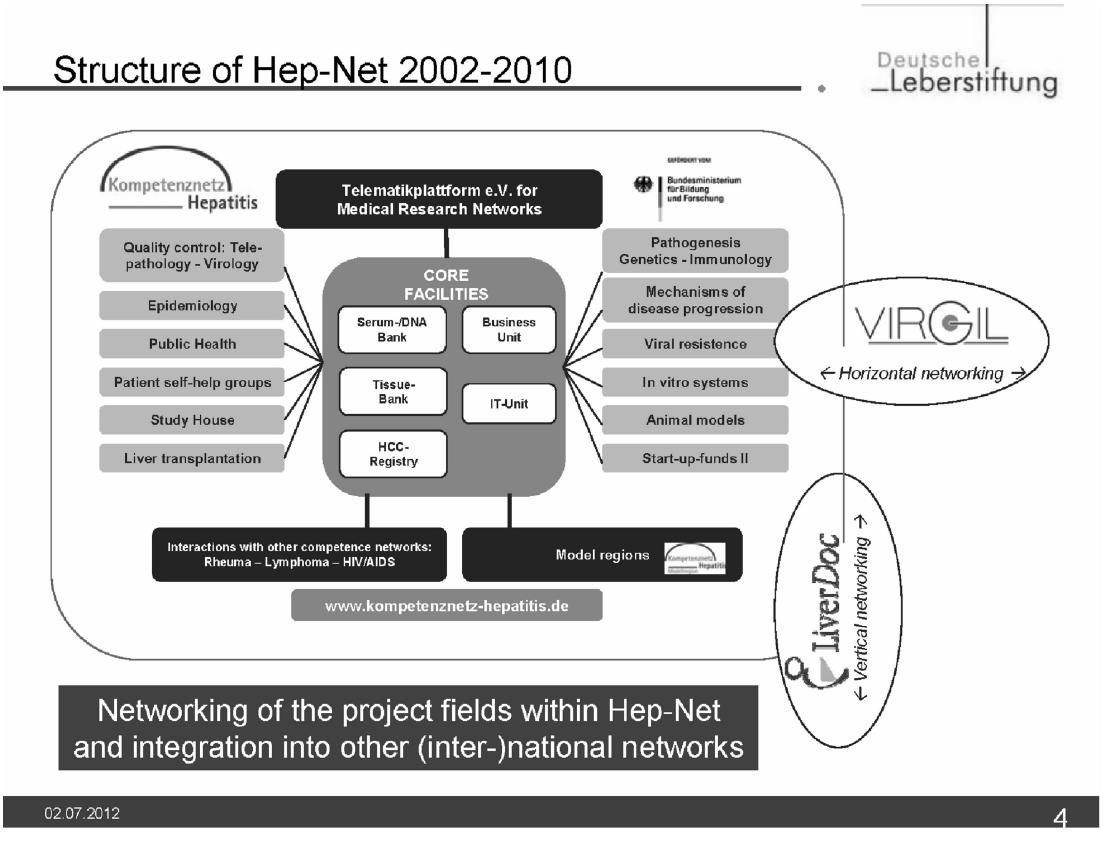
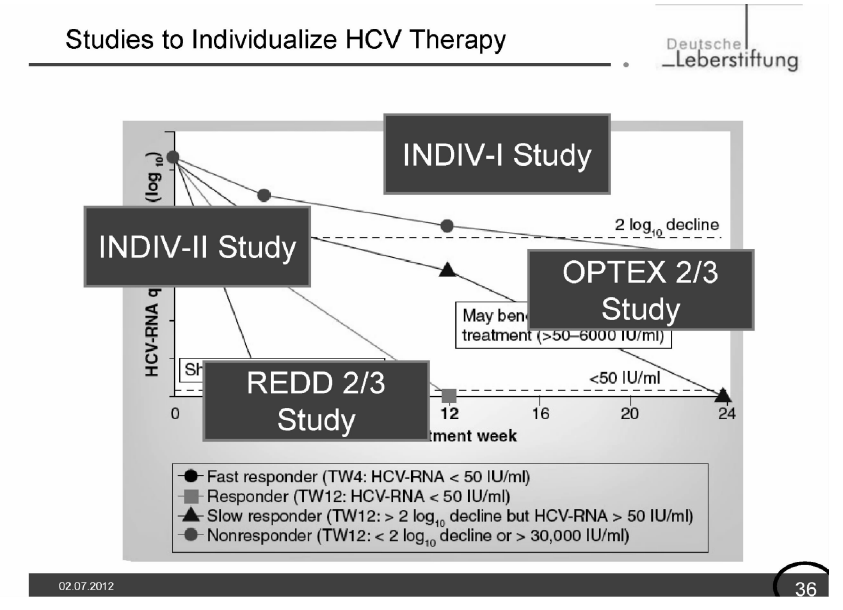
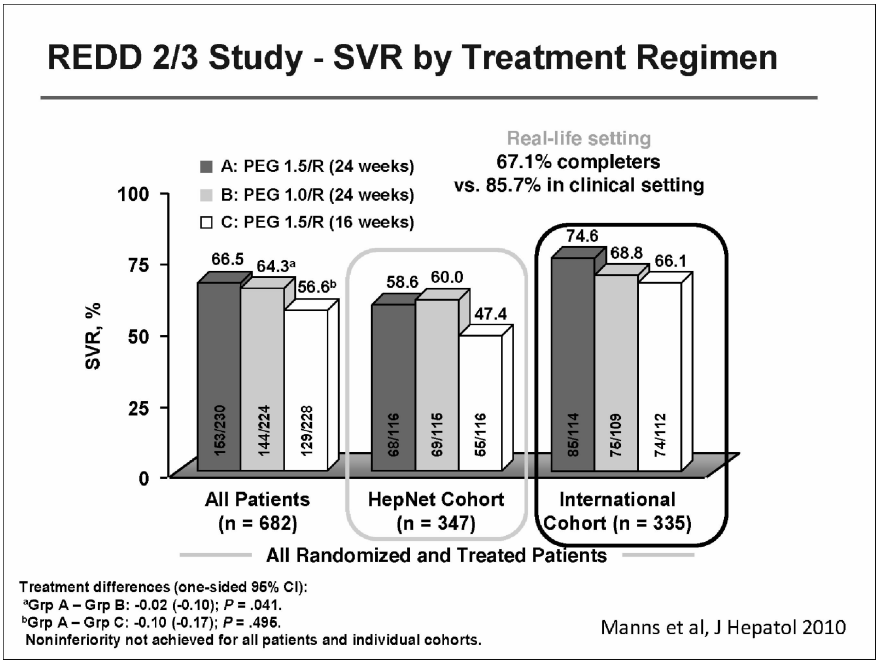
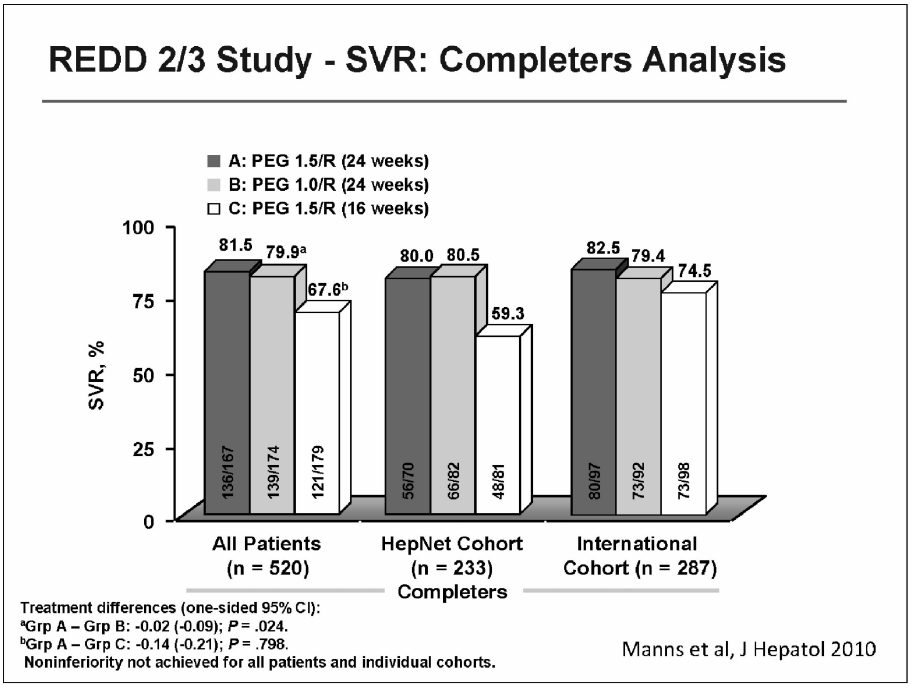


Fig. 2 The structure of the network

guidelines. In addition, we collaborated with the pharmaceutical industry to fulfil postapproval commitments, e. g. for PEG – IFN plus ribavirin therapies in HCV genotypes 2 and 3. This REDD 2,3 study was the first Hep Net study that established international networking of the German network.


Studies to Individualize HCV Therapy
Deutsche Leberstiftung
INDIV-I Study
INDIV-II Study
OPTEX 2/3 Study
REDD 2/3 Study
2 log10 decline
<50 IU/ml
Fast responder (TW4: HCV-RNA < 50 IU/ml)
Responder (TW12: HCV-RNA < 50 IU/ml)
Slow responder (TW12: > 2 log10 decline but HCV-RNA > 50 IU/ml)
Nonresponder (TW12: < 2 log10 decline or > 30,000 IU/ml)
02.07.2012
36


REDD 2/3 Study - SVR by Treatment Regimen
Real-life setting
67.1% completers
vs. 85.7% in clinical setting
A: PEG 1.5/R (24 weeks)
B: PEG 1.0/R (24 weeks)
C: PEG 1.5/R (16 weeks)
SVR, %
66.5
64.3a
56.6b
58.6
60.0
47.4
74.6
68.8
66.1
153/230
144/224
129/228
68/116
69/116
65/116
85/114
76/109
74/112
All Patients (n = 682)
HepNet Cohort (n = 347)
International Cohort (n = 335)
All Randomized and Treated Patients
Treatment differences (one-sided 95% CI):
aGrp A – Grp B: -0.02 (-0.10); P = .041.
bGrp A – Grp C: -0.10 (-0.17); P = .496.
Noninferiority not achieved for all patients and individual cohorts.
Manns et al, J Hepatol 2010


REDD 2/3 Study - SVR: Completers Analysis
A: PEG 1.5/R (24 weeks)
B: PEG 1.0/R (24 weeks)
C: PEG 1.5/R (16 weeks)
SVR, %
100
75
50
25
0
81.5
79.9a
67.6b
80.0
80.5
59.3
82.5
79.4
74.5
136/167
139/174
121/179
56/70
66/82
48/81
80/97
73/92
73/98
All Patients (n = 520)
HepNet Cohort (n = 233)
International Cohort (n = 287)
Completers
Treatment differences (one-sided 95% CI):
aGrp A – Grp B: -0.02 (-0.09); P = .024.
bGrp A – Grp C: -0.14 (-0.21); P = .798.
Noninferiority not achieved for all patients and individual cohorts.
Manns et al, J Hepatol 2010

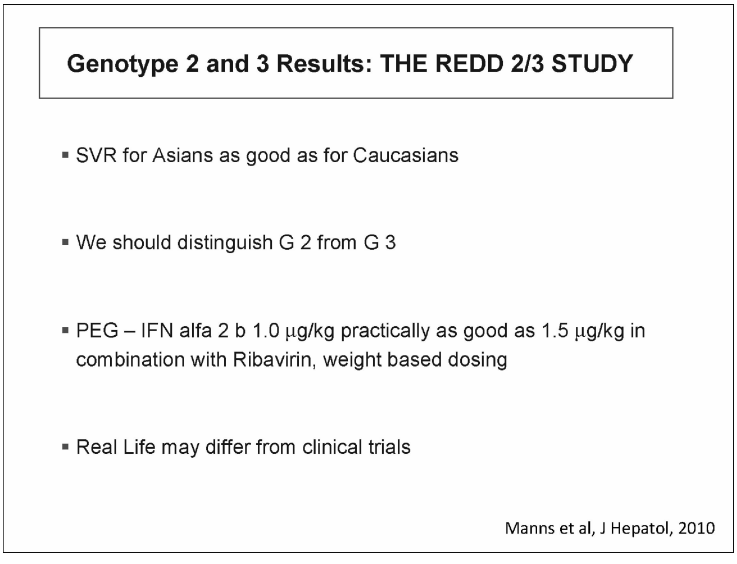
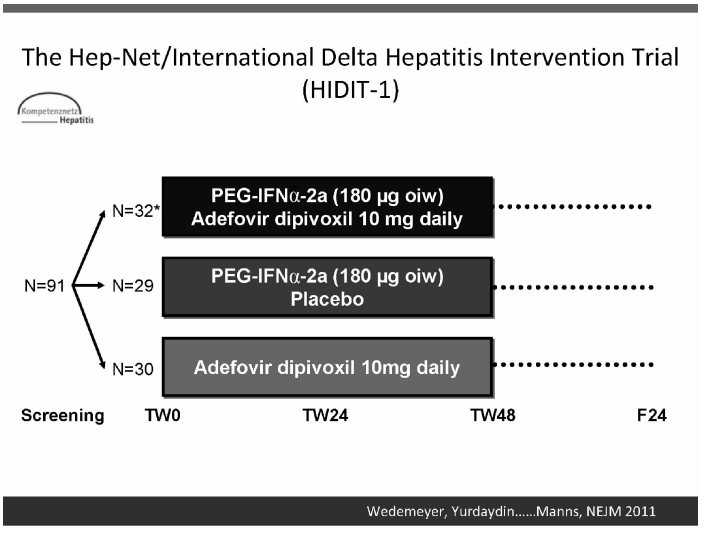

Genotype 2 and 3 Results: THE REDD 2/3 STUDY
SVR for Asians as good as for Caucasians
We should distinguish G 2 from G 3
PEG – IFN alfa 2 b 1.0 μg/kg practically as good as 1.5 μg/kg in combination with Ribavirin, weight based dosing
Real Life may differ from clinical trials
Manns et al, J Hepatol, 2010

Fig. 3



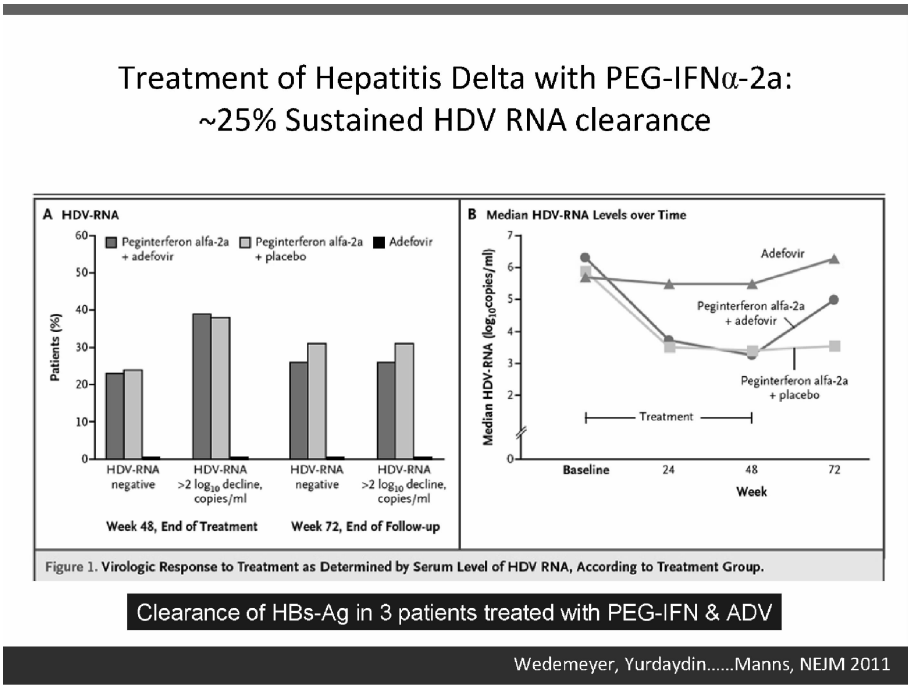


Fig. 4

Finally, Hep Net performs therapeutic multicenter and multinational trials for innovative therapies in hepatitis D (Delta). The first trial, HIDIT I, compared 48 weeks of PEG – IFN versus adefovir versus PEG – IFN plus adefovir combination therapy. Centers from Germany, Turkey and Greece participated in the study. In the present socalled Hep Net HIDIT Ⅱ trial, 96 weeks of PEG – IFN α – 2a monotherapy are compared with a combination of PEG – IFN plus tenofovir. This HIDIT Ⅱ study is fully enrolled and is running in Turkey, Greece, Rumania and Germany. Various substudies

have been performed by Hep Net investigators taking advantage of these large multicenter trials and the experimental expertise of Hep Net scientists contributing to our understanding of pathogenesis and therapy in viral hepatitis.

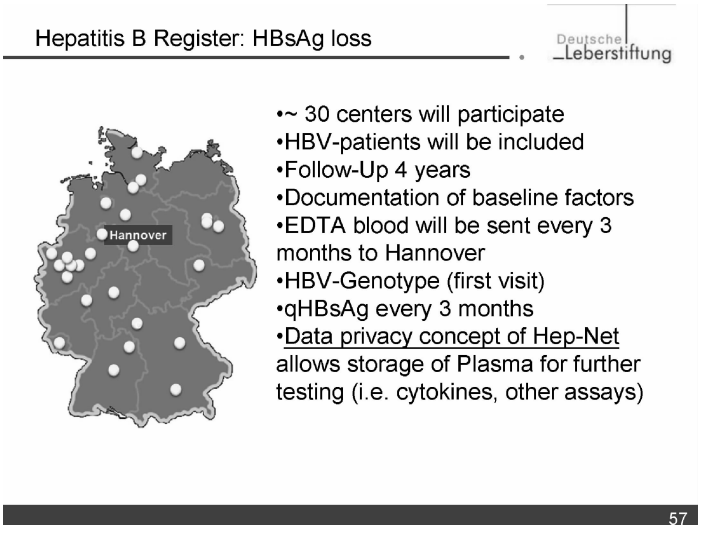
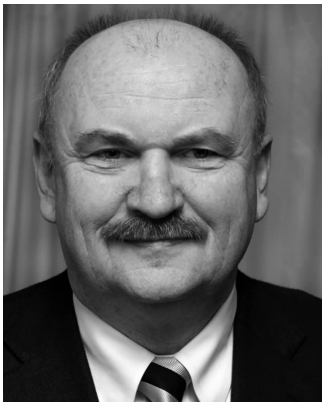


Fig. 5 Hepatitis B Register: HBsAg loss

Fig. 6 German Center for Infection Research



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Since 2006 Founder and President, German Liver Foundation;

2006 President, German Society of Gastroenterology (DGVS);

Since 2011 Member of the governing board, German Society for Internal Medicine (DGIM);

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Research fields: inflammatory liver diseases, viral and autoimmune, clinical trials for the treatment of liver diseases, in particular viral hepatitis, autoimmune liver diseases, liver transplantation, gastrointestinal oncology, in particular hepatocellular carcinoma, cell therapy: hepatocyte transplantation, regenerative medicine, mucosal immunology, clinical pharmacology.

The Epidemiology of Hepatitis B and C and Their Control Strategies in China

Yu Wang

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Hepatitis B virus (HBV) infection and its related diseases has brought serious harm to Chinese people and caused huge disease burden. HBV infection is highly endemic and a part of the patients can develop into chronic infections. According to a national epidemiological sero-survey, the prevalence of HBsAg was 9.75% in 1992.

Hepatitis B vaccine (HepB) is very effective to prevent HBV infections. In late 1980s, China started to use plasma-derived HepB, but due to the limited supply, HepB was mainly used in areas with high HBsAg prevalence, especially among those living in regions of high incidence of liver cancer. HB vaccination provided early protection against liver cancer for those people. Since the early 1990s, when the supply of yeast-derived HepB was sufficient, the government recommended and encouraged newborns to be vaccinated at the patients' expense. However, due to the differences in economic condition among provinces and between urban and rural areas, the coverage rate was high among residents in urban cities of developed areas but was low in poor and rural areas.

In 2002, HepB was integrated into China's Expanded Program on Immunization (EPI) and vaccines were provided free of charge, while only the users' fee and cost of syringes were charged to those who received the vaccine. This policy increased the coverage rate of vaccinee markedly. Since 2005, HepB was fully integrated into national immunization programm, and all costs related to HB vaccination were free of charge. Almost all newborns in China received timely-birth dose vaccination depending on the availability of health services in different regions. In 2006, the Ministry of Health of China conducted a national sero-epidemiological survey of HBV among the population aged

1–59. The survey showed that the prevalence of HBsAg among the population aged 1–59 was 7.18%, the lowest was only 0.96% in the age group 1–4, which had reached the goal of hepatitis B control set by WHO Western Pacific Region Organization, while the highest was in the age group 15–59, which was 8.57%.

Based on the sero-survey data in 1992 and 2006, it was estimated that the number of HBsAg carriers decreased by 19 million during the years, and 80 million children were protected from HBV infection. This brought tremendous social and economic benefit. The three-dose vaccine coverage rate of HepB ($HepB_3$) increased from 30% in 1992 to 93% in 2005, when first recommended HepB integrated into routine immunization management. The HepB Timely Birth Dose rate increased from 22% in 1992 to 82% in 2005. We may expect that when this generation reaches the child-bearing age, their offsprings would step into the era of hepatitis B elimination.

Although HepB will finally eliminate hepatitis B and HB associated diseases including chronic hepatitis B, it is expected that liver cirrhosis and HCC which are caused by HBV may also be controlled, we still have a long way to go. The decline of HBsAg prevalence among the general population predominantly depends on the protection of newborns by vaccination, which dilutes the numbers of current HBV infections and results in decrease of HBsAg carrier rate in the whole population. The fact which cannot be neglected is that currently there are still 93 million HBsAg carriers, 1 million cirrhosis, 350,000 HCC in China in 2010. This situation may last for many years and thus viral hepatitis B still is one of the highest disease burdens in China.

In general, there is a positive association between viral load of HBV and liver damage among carriers. While there is no therapeutic method to clear HBV and cure the patients, anti-viral treatment which can decrease the viral load in the blood becomes the current preferred therapy. So far, treatment with anti-viral drugs is in good progress and approximately 7–8 kinds of anti-viral drugs are available in clinical application. Due to the different effects of anti-viral drugs, the cost for treatment varies greatly. For the huge number of patients who need long-term treatment, China should organize studies and evaluation of the efficacies of anti-viral drugs in Chinese chronic hepatitis B patients. Development of evidence-based therapeutic guidelines and provision of experienced advice will greatly improve therapeutic efficacy, decrease medical cost and gain maximum benefit.

Hepatitis C is mainly transmitted by contaminated blood and blood products. Since

mid 1990s when China began to impose strict screening of blood supply, new cases of HCV infections became less. Recently, in drug abusers, co-infections of HCV, HIV and HBV were reported, which increase the incidence of new HCV infections. At the same time, due to the lack of education and control of country doctors in poor and rural areas in certain part of the country, the risk of transmitting HCV by contaminated medical devices increased. Recently, in the rural areas of Henan, Anhui, Guangdong provinces, clustered infection of HCV mainly among children and adolescents occurred, which indicates the urgent need for properly trained country doctors. Therefore, it is emphasized that together with financial support for modern equipments, to reinforce the training and education of country doctors and improve the quality of primary health care services are of priority. This will be an effective measure for the control of infectious diseases.

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Education and Career Background

Nov. 1991 – Oct. 1993

PhD, Department of Preventive Biology, Jichi Medical School, Japan

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Clinical and Research Interest

Viral hepatitis and related liver diseases

The Future of Research and Development of Vaccines against Viral Hepatitis

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Viral hepatitis is viral infection of hepatocytes as the major host cells. This group of viruses includes hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV), etc. From a public health point of view, hepatitis A, hepatitis B, hepatitis C and hepatitis E can be prevented and controlled by vaccines. Vaccines in R&D for viral hepatitis include preventive vaccine (PV) and therapeutic vaccine (TV). Preventive vaccine is required for controlling HAV, HBV, HCV or HEV, while therapeutic vaccine is mainly for HBV and HCV.

To date, PVs for HAV, HBV and HEV have been successfully developed. Phase I clinical trial of PV for HCV has been completed. Phase Ⅱ or Ⅲ clinical trials of TVs for HBV have also been completed. PVs for HCV are now undergoing phase I or Ⅱ clinical trials. In view of prevention and control of viral hepatitis, what kinds of vaccines are required? Which vaccines as priorities should be developed? Here I will provide some opinions on the current situations, problems, trends and strategies for R&D of viral hepatitis for discussion.

1. HAV vaccine

Hepatitis A is caused by hepatitis A virus and clinically characterized as acute hepatitis with jaundice. Normally hepatitis A can be cured and will not develop chronicity. Most cases of HAV infection in children are sub-clinical, while adult cases are acute. HAV, belonging to picornaviridae, is highly infectious and is transmitted through fecal-oral route. Both mucosal immunization and serum neutralization antibodies can block entry of HAV into hepatocytes. Specific cellular immunity can also efficiently clear the virus and

promote recovery.

1) Vaccine demands. PV is the most effective way to prevent and control hepatitis A, while TV is instead not necessary.

2) Current status. Preventive vaccines for hepatitis A consist of two types of vaccines: inactivated vaccines composed of inactivated wild-type strains; live attenuated vaccines prepared by viruses being passaged in cells to reduce infectivity.

3) Challenges. Inactivated vaccines are safe to use, with good efficacy, but rather expensive. In addition, there are bio-safety issues during production of inactivated vaccines when using wild-type or partially attenuated strains. Live attenuated vaccines are with good safety and efficacy, also relatively inexpensive, but it needs complicated facilites to store and transport attenuated vaccines. Furthermore, there are potential risks of recombination and reversion to wild type infectious strains.

4) Strategies. Currently, the two types of vaccines are basically able to meet the demand for prevention and control of hepatitis A, but HAV vaccine should be further improved in accordance with social and economic progresst. ① R&D of molecular identified attenuation of live attenuated vaccines, which can accurately remove the infectivity-related genes and solve the problems of reversion. ② Preparation of inactivated vaccines using stable attenuated strains; Solving the potential risks of bio-safety caused by using wild-type strains and putative reversion problem caused by using live attenuated vaccines. ③ Development of genetic-engineered VLP subunit vaccines to replace inactivated and live attenuated vaccines. ④ Development of polyvalent combined vaccines such as polyvalent inactivated vaccines, polyvalent live attenuated vaccines, and polyvalent subunit vaccines.

2. HBV vaccine

Hepatitis B is caused by HBV and is characterized by hepatitis with non-typical jaundice. Most perinatal infections caused by HBV become chronic virus carriers and among them some will progress to chronic hepatitis. In contrast, infections of adults can usually be cured. HBV is a member of hepdnaviridae and is not stable when exposed in environment. HBV is highly infectious and is mainly transmitted by blood, sex, and maternal-neonatal transmission. Neutralization antibody in serum can block virus infection of hepatocytes, while induced cellular immunity can clear the virus and promote recovery.

1) Vaccine demands. PV is the most effective tool for prevention and control, and TVs can be of help for the recovery of chronic hepatitis B patients.

2) Current status. ① PVs have achieved a great success. Genetic-engineered vaccines (produced in yeast and CHO cells) are widely used for the prevention of hepatitis B to replace the previous plasma-derived vaccines. ② TVs. In China, there are three TVs under clinical trials. The first is an antigen-antibody complex vaccine (phase IIIa trials is completed); the second is synthetic peptide vaccine (phase IIa trials are being conducted); the third is DNA vaccine (phase I trials are completed).

3) Challenges. ① PVs: Highly safe with good efficacy and low cost. Approximately 3% –10% of vaccines are non-responsive or lowly responsive. ② TVs: Clinical efficacy is yet not fully satisfactory. The vaccine candidates used in clinical trials are limited. In China, there are totally 3 TVs, among which 2 are under phase Ⅱ—Ⅲ trials.

4) Strategies. ① Currently, PVs basically can meet social demands, but in view of marketing, it is necessary to solve the issues of low-responders or non-responders. Meanwhile, it is important to either exploit high dose (60 μg/dose) of HBV vaccine or develop vaccines with multiple antigens, like preS1. ③ There are urgent needs for TVs, but development of TVs is still at the stage of concept-study. Therefore, basic studies on approaches to break immune tolerance of HBV should be strengthened and phase Ⅰ—Ⅱ clinical trials based on distinct ideas and design strategies should also be encouraged. ④ Study on combined use of TVs and therapeutic drugs.

3. HCV vaccine

Hepatitis C is caused by HCV. Acute cases do not show severe clinical manifestations and jaundice. In acute hepatitis C cases, ~20% of patients will clear the virus and recover, while the rest will develop chronic infection, which is an important cause for chronic hepatitis. HCV is a single-stranded positive RNA virus and belongs to flaviviridae. HCV is transmitted by blood, sex and maternal-neonatal transmission. As 20% of acute HCV cases could be self-limited, it suggests there is the possibility to develop HCV vaccine. It will be much difficult to develop HCV vaccine, because HCV is highly mutagenic and most HCV cases will progess to chronicity.

1) Vaccine demands. ① To date, combination of therapeutic drugs has achieved excellent efficacy, but it remains important to develop a safe and potent HCV vaccine to control HCV infections (especially for the high-risk population). ② Similarly,

development of therapeutic HCV vaccine will have practical significance to improve the efficacy of current drugs, to decrease the costs of drug therapy and deal with the possible drug resistance.

2) Current status. No HCV vaccine is yet on the market. Phase I clinical trials on PV using virus vector, VLP vaccine are ongoing. Phase Ⅰ—Ⅱ clinical trials on TVs are also ongoing.

3) Challenges. PVs and TVs are still at the stage of concept study. Some experiments in animals suggest that these vaccines have immune-protective effects, but the whole R&D route has not yet been completed.

4) Strategies. ① PVs: Strengthen studies on the mechanisms of immune protection in naturally recovered acute patients. Because HCV is highly mutagenic, HCV vaccine should basically be of broad-spectrum. It is especially important to do the study of confirming broad-spectrum cross neutralization antibody and broad-spectrum cross cellular immunity. ② TVs: Reinforce basic studies on effective therapeutic mechanisms of chronic infection of HCV. Encourage phase Ⅰ—Ⅱ clinical trials on TVs. Emphasize studies of combined application of TVs and therapeutic drugs. ③ Generate new ideas: It is difficult to achieve success based on traditional theories and strategies of vaccine development, because HCV is highly mutagenic and easy to cause chronic infection. Therefore, it is crucial to generate innovative ideas for developing vaccines against hepatitis C.

4. HEV vaccine

Hepatitis E is caused by HEV and characterized by the manifestation of acute hepatitis with jaundice. Hepatitis E is mainly transmitted by the intestinal route. HEV is also transmitted by transfusion and vertical transmission. Approximately 1/3 population of the whole world have been infected by HEV, but most cases are sub-clinical infection. Hepatitis E cases are mostly sporadic and also endemic. Acute cases can be self-limited. HEV is single-stranded positive RNA virus and belongs to hepevirus genus of hepeviridae. HEV has only one serotype. Although a number of HEV infection animal models have been identified, except under unique conditions, HEV cannot be cultured *in vitro*. Therefore, HEV vaccine can only be developed via the genetic-engineering technology. Both neutralization antibodies in serum and mucosal immunity can block HEV infection of hepatocytes.

1) Vaccine demands. In the view of disease prevention and control, development of HEV vaccine is important. For hepatitis E, TV is not necessary.

2) Current status. Phase Ⅲ clinical trials of HEV vaccine, which was composed of virus like particles (VLP) prepared by HEV ORF2 protein expressed in *E. coli*, have been completed and have shown good preventive effects. This vaccine has been approved and been on Chinese market in Dec 2011.

3) Challenges. Analysis of a large scale of mass vaccination remains to be studied because HEV vaccine has just been on the market.

4) Strategies. ① Promotion of vaccine to be used in highly prevalent regions and further observation of immunization efficacies and safety as well as analysis of cost-effectiveness. ② Development of polyvalent subunit vaccines for HEV and promotion of HEV vaccine use.

R&D of vaccine for viral hepatitis is a miniature reflecting R&D of traditional vaccines and genetic-engineered vaccines in the past 3 decades. On the one hand, the history (e.g. R&D of live attenuated and inactivated vaccines for HAV and plasma-derived vaccine for HBV) represents the value and application of traditional technology in R&D of vaccine. On the other hand, it also reflects the valuable and indispensible roles of genetic-engineered vaccines in developing novel vaccines. Genetic-engineered HBV vaccine is a good model of improving or replacing the traditional vaccines (plasma-derived vaccines). HEV vaccine also developed by genetic-engineered technology represents a novel one that could not be achieved by traditional technology. In addition, R&D of HCV vaccines and HBV therapeutic vaccines reflect that perhaps only genetic-engineered technology could lead to the success to develop novel vaccines for viral infections, especially when targeting multi-genotypes of viruses, which are prone to mutate, and can lead to persistent infections.

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Professor of Biotech Center for Emergency Response of Viral Diseases in Institute for Viral Disease Control and Prevention, China CDC.

Dr. Li Ruan is a professor of Biotech Center for Viral Disease Emergency Response in Institute for Viral Disease Control and Prevention, China CDC. He was the chairman of Biotech Center for Viral Disease Emergency Response in Institute for Viral Disease Control and Prevention in 1992 – 2008 and the director of this Institute in 1997 – 2004. He used to work in Department of Microbiology of Mt. Sinai School of Medicine in New York of US with Dr. Peter Palese as visiting scientist during the period of 1984 to 1986 and worked with Dr. Max Essex for two months in Harvard School of Public Health in Boston of US in year 2000.

Dr. Li Ruan has been working in the fields of Virology, Viral Disease Control and Prevention and vaccine research and development since 1980. He developed three kinds of gene expression system including mammalian cell system, virus system (such as vaccinia virus Tian Tan strain and adenovirus) and aspergillus fungi system. He made very important contribution in the development of recombinant hepatitis B vaccine in China. He was in charge of development of polyvalent vaccine against HAV, HBV, RSV, measles and encephalitis, and therapeutic vaccine against HBV. He also takes part in research of therapeutic vaccine against EBV and HPV. Five vaccinia virus recombinants developed by him were approved by Chinese SFDA and had completed phase I trials in 1990s. He was the principal investigator for research of HPV vaccine granted by EU during 1996 – 2000 and for development of China HIV/AIDS vaccine in CIPRA program granted by NIH of US during 2002 – 2007. In last 20 years, he has been involved in publication of more than 10 books, and has published nearly 200 articles. Furthermore, he has applied for eleven patents. He received five prizes of National Science and Technology Progress Award.

The Urgent Need to Control Hepatitis C with Vaccines, New Therapeutics and Public Education

Michael Houghton

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The WHO estimates that there are up to 170 million people carrying the hepatitis C virus (HCV) globally, with several million new infections occurring each year. Since up to 20% of infected individuals will develop cirrhosis typically over the course of decades and with about 5% eventually developing hepatocellular carcinoma, there is a very urgent need to implement controlling measures, especially in countries like China where there is a relatively high prevalence of infection[1]. Firstly, these measures should comprise informing the general public of the risk of acquiring hepatitis C through exposure to contaminated blood which can be via re-use of needles and syringes, unhygienic tattooing or ear- or body-piercing and any traditional medical or other practices involving blood transfer. Secondly, the new HCV protease inhibitors were approved for human use in North America in 2011 and together with interferon-alpha and ribavirin can now cure about 70% of HCV carriers[2]. Making these new drugs available is obviously of great urgency. Many new drugs inhibiting the HCV polymerase, the HCV nonstructural protein 5a and other viral targets are in advanced clinical development and when combined with each other and/or with existing drugs, offer the real possibility of curing all treated patients[2]. As in HIV therapy, combination therapy is needed in order to avoid the ready emergence of drug-resistant virus[2]. It is important that all countries prepare economically and logistically for the advent of these effective but expensive drug combinations. Effective therapy will also require sophisticated clinical management of patients, including monitoring for drug resistance. In Canada and elsewhere, outreach to obscure geographical locations has been successfully achieved through clinical forums run on the internet, thus enabling the efficient sharing of crucial

information and expert clinical advice. Another new area of great promise is the use of oncolytic viruses to treat liver cancer. This disease has had very limited therapeutic options to date but the use of attenuated viruses to target and kill cancer cells specifically is emerging as a key medical area. A recently-reported phase 2 clinical trial in the USA has reported a doubling in the progression-free survival time of patients with hepatocellular carcinoma by the repeated administration of an easily-grown, attenuated vaccinia virus, previously used in the small-pox vaccine[3]. Many other types of promising oncolytic virus are also in clinical development for liver and other cancers.

Despite historical pessimism about the prospects for an HCV vaccine, there is now genuine optimism for developing successful HCV vaccines. We now know that there is natural immunity acquired against HCV infection, that this immunity correlates with protective $CD4^+$ & $CD8^+$ T cell responses against the virus and is also associated with the early production of cross-neutralising antibodies against the virus, and that it is possible to significantly reduce the incidence of the chronically-infected carrier state in animals that were vaccinated prior to experimental challenge[4]. A phase 2 prophylactic clinical trial is ongoing in the USA using defective adenoviruses expressing the nonstructural genes 3, 4 & 5 from a 1b subtype predominant in China[5]. This vaccine is likely to provide valuable, partial protection but it is possible that its efficacy could be further enhanced by also eliciting cross-neutralising antibodies to the many clades of HCV. My laboratory has shown recently that the latter is feasible in vaccinated humans. Finally, new data reported at the 2012 EASL meeting by Transgene and collaborators indicate that a vaccine against HCV has therapeutic value when combined with the antivirals, pegylated interferon-alpha plus ribavirin. At week 12 after the initiation of therapy, twice as many patients were free of virus compared with those patients receiving the antivirals alone without the vaccine[6]. This vaccine is relatively simple to produce using the defective MVA version of vaccinia virus to express HCV nonstructural proteins 3,4 & 5.

In conclusion, there has been great progress made toward controlling HCV infection which can be achieved through 1) Greater public education about risk factors for infection, 2) The implementation of the new HCV drugs inhibiting the viral protease, polymerase, NS5a and other viral targets as well as a therapeutic HCV vaccine & 3) Implementing a prophylactic vaccine program that stimulates both cell-mediated

immunity to the virus as well as cross-neutralising antibodies to the multiple strains of HCV.

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Michael Houghton, PhD, is the Li ka Shing Professor and holder of the Canada Excellence in Research Chair (CERC) in Virology within the Li Ka Shing Institute of Virology at the University of Alberta, where he is focusing on research into viral hepatitis and other inflammatory diseases. Together with Dr. G. Kuo & Dr. Q-L. Choo at the Chiron Corporation and Dr. D. Bradley at the CDC in the USA, he discovered the hepatitis C virus, developed blood tests and identified new drug targets for this virus as well as developing a vaccine for clinical development. His laboratory was also the first to characterize the molecular propertiy of viral hepatitis D genome and

the human beta-interferon gene. He and colleagues have received numerous awards for their work on hepatitis C including the Clinical Lasker and Karl Landsteiner awards from the USA, the Robert Koch Medal from Germany and the Gold Medal from the Canadian Liver Disease Association. He is an author of more than 200 research publications.

Future Trends in Liver Disease Affecting Clinical and Epidemiological Outcomes

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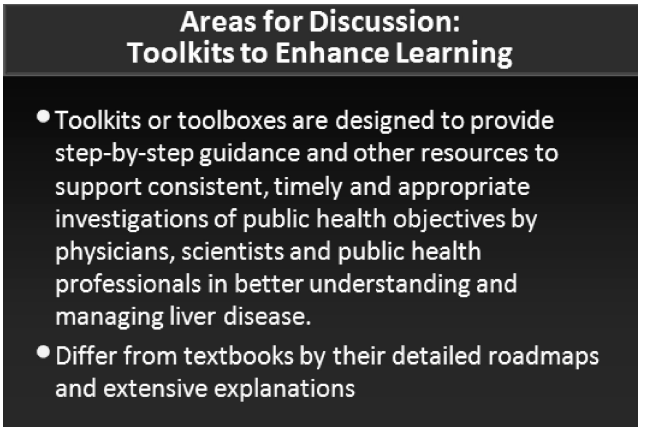
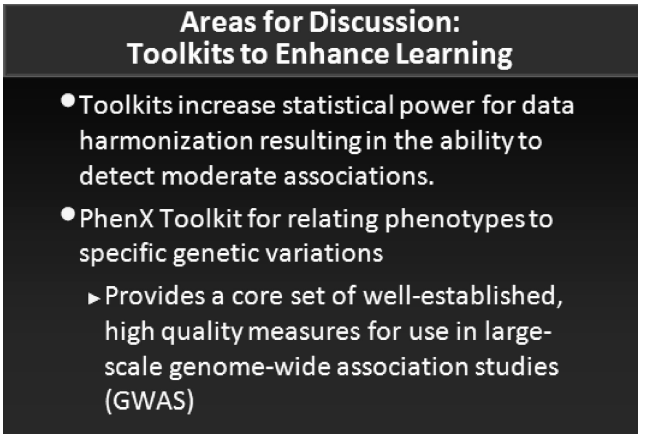
This article is designed to discuss how we, as hepatologists, will navigate through the clinical and epidemiological aspects of liver disease over the next two decades. Trying to forecast the future, even twenty years in advance, can be a daunting task. Laozi, a 6th century BC Chinese poet said "**Those who have knowledge don't predict. Those who predict don't have knowledge.**" The one thing about predictions is that they are either right or they are wrong. According to some scientists, the future will be exactly like the past, only far more expensive.

Three areas have been selected that might be fruitful for discussion

First, it is clear that hepatologists and gastroenterologists are not going to be able to manage all cases of liver disease, but may be able to co-manage patients with surrogate nonspecialists such as those practicing Family Medicine or Internal Medicine or physician extenders such as physician assistants or nurse practitioners. This would have high societal impact on health and economics and would avert serious outcomes of untreated disease. Its goal would be to link primary care with knowledge networks through the use of technology such as video- and teleconferencing, smart phones and the web to educate and assist primary care physicians ("intensivists") in the recognition and management of uncomplicated cases of liver disease for the purpose of improving outcomes. This can be accomplished by reducing variations in the care of patients by sharing best practices based on consensus guidelines. The mission would be to expand care for liver disease patients in underserved areas, and to continuously monitor outcomes through ongoing interactions between intensivists and hepatologists. It

is expected that the benefits to the health care system utilizing this approach will be considerable. Networking would reduce variation in care, while knowledge would be transferred and reinforced. Real-time consultations, available 24/7, that are facilitated by weekly telemedicine clinics attended by hepatologists, radiologists, and oncologists to discuss cases and to educate and mentor community physicians would provide the infrastructure for the program. Professional satisfaction would be improved bilaterally through learning loops, and care would be cost-driven by avoiding excessive testing and travel. Effectiveness of the program would be assessed through the installation of a HIPAA compliant web-based database. Referral to the specialist would only be necessary when the complexities of care demand such a transfer of services.



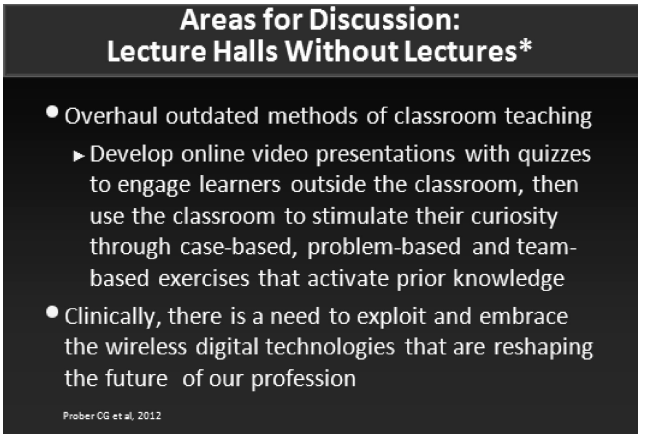


In the USA, such a program already is achieving a modicum of success. The project is called ECHO (Extension for Community Healthcare Outcomes) and is the creation of Dr. Sanjeev Arora, M. D. at the University of New Mexico Health Sciences Center in Albuquerque, New Mexico[1,2]. It is a model to bring high quality care to very

sick patients wherever they live. The ECHO model has expanded health care to thousands of patients, many from isolated and poor communities. The primary care intensivists receive instructions in how to care for complex liver disease patients. As a result, many patients are living longer, healthier lives with less pain and disability. A pilot project has recently been launched for treating community-based chronic hepatitis C[3,4]. Initial assessment of the program has shown comparable quality of care and SVR rates that did not differ significantly between patients managed by the university and those managed by primary care physicians. The more complex cases are referred to the specialists. This innovative model has the potential to reduce program expenditures while preserving or enhancing the quality of care. An important issue is how to convince the health care industry of the value of this type of program, both medically and financially, and this has become the focus of the debate. Will the patient receive the same quality of care as they might from a specialist? Probably not, but the resilience of patients and the identification and treatment of liver disease at an early stage before irreversibility or complications ensue clearly favors this unique format.

Another current and future challenge for hepatologists is creating a proper learning environment for studying the clinical and epidemiological aspects of liver disease. This can be accomplished by designing and implementing toolkits for disease management and epidemiologic investigations so that data is not compromised. How often have we seen a meta-analysis performed on a topic only to witness many of the studies being censored because data from the various studies could not be assembled in meaningful manner? One example of how a toolkit works to facilitate research being conducted by a number of independent investigators is the PhenX Toolkit for comparing phenotypes and exposures across studies. This toolkit is designed to provide "a core set of well-established, low burden, high quality measures for use in large-scale genomic studies."[5,6] This project was an outgrowth of the rapid progress being made in genomic technology that has led to numerous genome-wide association studies (GWAS) that are designed to relate phenotypes to specific genetic variations. Thousands of genetic polymorphisms can now be analyzed increasing our understanding of disease etiology and pathogenetic mechanisms leading to the development of novel prophylactic or therapeutic agents. To facilitate rapid and valid measurements across studies, RTI International in Research Triangle Park, North

Carolina, and the National Human Genome Research Institute are collaborating with working groups of domain experts that includes input from the scientific community using a consensus process. RTI is an independent, nonprofit institute whose "mission is to improve the human condition by turning knowledge into practice." Broad acceptance of these measures is expected to increase statistical power for data harmonization resulting in the ability to detect moderate associations. The toolkit also includes the incorporation of other potential contributing factors such as environmental exposures (ambient environment, personal behaviors, treatments). By capturing these data in well-defined formats, generating reports, and sharing the contents with colleagues, subsequent meta-analyses can lead to the discovery of new gene associations. These measures are available to the scientific community via the Web-based toolkit. Gastrointestinal and Infectious Diseases and Immunity are two of the 20 research domains being delineated by the project, either one of which could be the source of an additional toolkit for hepatology.



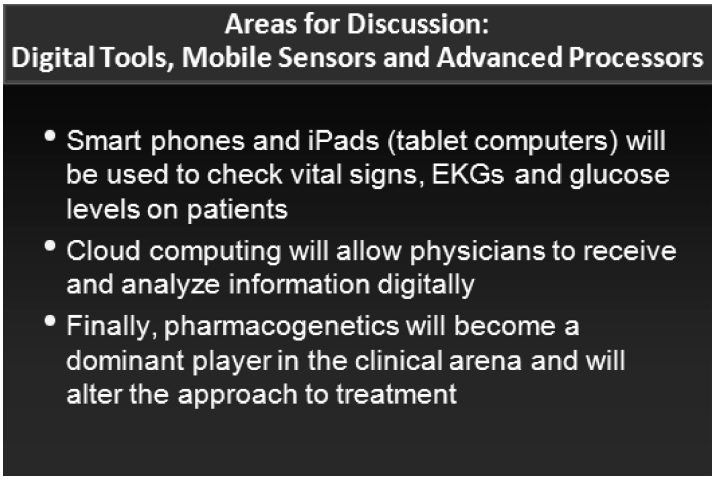
One organization that has a free, online collection of local public health tools produced by members of the public health community is the National Association of County and City Health Officials (NACCHO)[7]. They have developed a number of toolboxes to inform and improve the work of public health professionals in the promotion and advancement of public health objectives. Among these toolboxes is one on "Adult Hepatitis B Virus (HBV) Vaccination: An Implementation Guide for Local Public Health."[8] The North East, North West, Yorkshire and Humber Regional Epidemiology Units have produced a Field Epidemiology Toolkit[9] designed to provide step-by-step guidance and other resources to support consistent, timely and appropriate investigations of infectious disease outbreaks and other incidents which may require a

field epidemiology approach. The World Health Organization (WHO) has an excellent toolkit on unsafe injection entitled "WHO best practices for injections and related procedures toolkit."[10] As for many of these toolkits, the guidance is generic making it applicable to a variety of situations. There is also an Evidence-Based Medicine Toolkit[11] and a toolkit on hepatitis A, B or C investigation guidelines[12]. A subcommittee of the Transfusion-Transmitted Diseases Committee for the AABB in the United States is developing a toolkit for emerging infectious disease agents that are a potential threat to transfusion safety utilizing previously published data by this group[13]. The Wisconsin Department of Health and Family Services have produced a Wisconsin Hepatitis Strategic Plan[14] to prevent, detect and control viral hepatitis. What set these toolkits apart from textbooks are their detailed and well-defined roadmaps. It is predicted that more of these toolkits will be introduced in clinical medicine over the next few years that can provide comprehensive, step-by-step approaches designed to assist physicians, physician extenders, and public health professionals in better understanding and managing liver disease.

In addition to these toolkits, it is anticipated that hepatologists, statisticians, and epidemiologists will unite to produce more in-depth courses on epidemiology such as the excellent program developed by the APASL, AASLD and EASL at the 2012 annual APASL meeting in Taiwan. Programs such as this need to be videotaped and promoted by making the sessions available online to hepatologists throughout the world. In addition, a central web site needs to be established and paid for by the government and/or the various hepatology organizations where experts can be contacted through Skype, by e-mail or by teleconferencing to provide guidance and practical experience to their membership in an ongoing program. To make it successful, responses must be real-time and available 24/7 similar to a Chat room with proper legal protection put into place.

The future of our discipline is changing rapidly. Outdated methods of teaching must give way to new technology in the way that we educate our students, residents and fellows. We need to consider developing 10 – 15 min online video presentations with embedded quizzes that engage the learners outside the classroom at their own pace by testing their comprehension of the lesson, and then use the classroom to stimulate their curiosity through case-based, problem-based and team-based exercises that will

activate their prior knowledge[15]. Studies have shown that such a format is conducive to learning and that students are hungry for such interaction. Clinically, we must exploit and embrace the wireless digital innovative technologies that are going to reshape the future of our profession[16]. There is a "cloud" over our heads and it has nothing to do with rainfall. Cloud computing will allow users to increase capacity or add capabilities on the fly without investing in new infrastructure, training new personnel, or licensing new software. It is the supercomputer we have always dreamed of owning. This can be entity-based with data shared only to the members.



In the next phase of medicine, physicians will utilize powerful digital tools, including mobile sensors and advanced processors, which will transform our understanding of an individual. Smart phones will be used to check vital signs, monitor heart rhythm and determine blood glucose levels. Physicians remote from the medical centers will be able to have information transmitted digitally. Already there is a handheld, pocket-sized visualization tool powered by ultrasound technology that enables you to visually inspect the inside of a patient's body during a physical exam including obtaining an echocardiogram. These data could be sent wirelessly to experts for assistance in interpretation. Embedded sensors could be programmed to sense for cancer cells in the blood or to detect circulating endothelial cells that are the harbinger of clot formation and heart attacks or the incipient development of thromboses. With the introduction of these information systems that utilize wireless sensors, there will be a shift to the doctor-patient relationship with the physician establishing a partnership with the patient in order to guide him. Pharmacogenetics will become a dominant player in the clinical arena. Clinicians already test patients for potential drug toxicity that may occur following the

administration of azathioprine by looking for mutant thiopurine methyltransferase (TPMT) alleles that may result in impaired metabolism leading to the development of severe bone marrow toxicity. Similarly, the benefit of testing for the IL28B polymorphism assists hepatologists in determining probabilities for success in the treatment of hepatitis C infection. In the United States, the top three prescription drugs are all TNF-α inhibitors, and these represent a 27 billion dollar industry. However, only 40% of individuals who are treated with these agents respond. Health insurance companies are very interested in determining who will respond and are sequencing responders for this information (see previous comments about the PhenX project). Thus, as pharmaceutical agents become more expensive, we can expect that the healthcare industry will be very interested in the outcome of such research. In the interim, there have been some innovative ideas. Sam Waksal, the former chief executive of ImClone who spent five years in a federal prison for insider trading and is now CEO of Kadmon Pharmaceuticals, has put together a provocative plan called the "pay-for-response model."[17] In this proposal, patients would only pay if they responded to a drug. For hepatitis C, this might mean achieving a sustained virological response or SVR. Those not exhibiting an SVR would not pay for the medication. He believes that this would not only save money, but would encourage pharmaceutical companies to determine the reasons for failure and how to resolve this. This might also achieve a paradigm shift where multiple drugs, perhaps from different companies, might be combined for greater success. This may be the future of biotechnology although it won't happen overnight. Ultimately, however, personalized medicine and targeted therapy will change how clinicians care for their patients.

In this short discourse, an attempt has been made to look into the future and to

provide limited insight into how discoveries in genetics and biology will revolutionize medicine as it is currently practiced and how these innovations can be utilized by hepatologists in the clinical and epidemiology arenas. We need to embrace novel technology and find new ways to analyze data more efficiently while continuing to improve the care of our patients.

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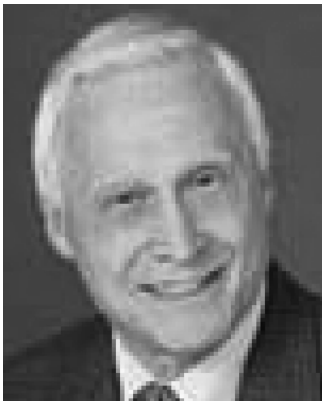

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Clinical and Research Interests

Viral hepatitis (all agents), autoimmune hepatitis, drug-induced hepatitis, primary biliary cirrhosis, metabolic liver diseases, alcoholic liver diseases, blood-borne pathogens,

immunopathogenesis of viral hepatitis, early stages of hepatitis C infection and virus structure, replication of hepatitis C virus in culture and small animal models, safety of the U. S. blood supply, occult hepatitis B and C infection.

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后　记

科学技术是第一生产力。纵观历史，人类文明的每一次进步都是由重大的科学发现与技术革命所引领和支撑的。进入21世纪，科学技术日益成为经济社会发展的主要驱动力。我们国家的发展必须以科学发展为主题，以加快转变经济发展方式为主线。而实现科学发展、加快转变经济发展方式，最根本的是要依靠科技的力量，最关键的是要大幅提高自主创新能力，要推动我国经济社会发展尽快走上创新驱动的轨道。党的十八大报告指出，科技创新是提高社会生产力和综合国力的重要支撑，必须摆在国家发展全局的核心位置，要实施“创新驱动发展战略”。

面对未来发展的重任，中国工程院将进一步发挥院士作用，邀请世界顶级专家参与，共同以国际视野和战略思维开展学术交流与研讨，为国家战略决策提供科学思想和系统方案，以科学咨询支持科学决策，以科学决策引领科学发展。

只有高瞻远瞩，才能统筹协调、突出重点地建设好国家创新体系。工程院历来高度重视中长期工程科技发展战略研究，通过对未来20年及至更长远的工程科技发展前景进行展望与规划，做好顶层设计，推动我国经济社会发展尽快走上创新驱动的轨道。

自2011年起，中国工程院开始举办一系列国际工程科技发展战略高端论坛，旨在为相关领域的中外顶级专家搭建高水平高层次的国际交流平台，通过开展宏观性、战略性、前瞻性的研究，进一步认识和把握工程科技发展的客观规律，从而更好地引领未来工程科技的发展。

中国工程院学术与出版委员会将国际工程科技发展战略高端论坛的报告汇编出版。仅以此编之作聚百家之智，汇学术前沿之观点，为人类工程科技发展贡献一份力量。

中国工程院