Role of glucose metabolism and FDG uptake in HCC biology

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²Shiga Medical Center Research Institute, Shiga, Japan
CQ12 In diagnostic imaging of hepatocellular carcinoma, are nuclear medicine techniques, including FDG-PET, more useful as compared with other imaging methods?

• RECOMMENDATION

• Conventional liver scintigraphy does not contribute to the diagnosis of hepatocellular carcinoma. (grade D)
• Fluorodeoxyglucose PET is no more useful for the diagnosis of the primary tumor than other conventional test methods. (grade C2)
• When extrahepatic metastasis is suspected but cannot be detected by other imaging methods, it would be useful to add FDG-PET for the evaluation. (grade B)
CQ16 Are brain MRI, chest CT, bone scintigraphy, and FDG-PET necessary for determining the stage of HCC?

**RECOMMENDATION**

- Chest CT, bone scintigraphy, and FDG-PET scans can be recommended for HCC patients with risk factors for extrahepatic metastases *(Grade B)*.

- FDG-PET is an excellent diagnostic tool for detecting extrahepatic metastasis from HCC, including bone metastases, and it may be appropriate to proactively perform FDG-PET in patients with elevated tumor marker levels that cannot be accounted for by the presence of abdominal lesions and/or lung metastasis alone.

**Is FDG-PET no more useful for diagnosis of the primary tumor in patients with HCC?**
Significance of PET on HCC

What can we get by PET in pts with HCC?

How do we have impact by PET on HCC management?
Uptake of FDG to Cancer Cells

Metabolic trapping
Accumulation of 18F-FDG as glucose analogue

18F-FDG

Membrane

18F-FDG

18F-FDG

Hexokinase

Glucose-6-phosphatase

Glucose

Glucose-6-phosphate

18F-FDG-6-phosphate

Glucose

Glucose-6-phosphate

Glycolysis

Via Glucose Transporter
FDG accumulation was analyzed quantitatively by calculating the standardized uptake value (SUV) in the regions of interest (ROI) placed over the tumor and the normal Liver. Standardized uptake value (SUV) = PET count \cdot \text{calibration factor} (\text{mCi/g}) where \text{Calibration factor} = \text{injection dose (mCi)/body weight (g)}

The ROI was 10 \cdot 10 \text{mm (independent of tumor size)} and was placed in tumor areas that exhibited the highest 18F-FDG activity.

\[
\text{SUV (standardized uptake value)} = \frac{\text{tissue activity concentration}}{\text{(injected dose / body weight)}}
\]

TNR (tumor to non-tumor SUV ratio) = \[
\frac{\text{tumor SUV}}{\text{non-tumor SUV}}
\]

Pancreatic cancer: $4.97 \pm 2.34$
Liver: $2.31 \pm 0.38$
Pancreas: $1.62 \pm 0.47$
### Sensitivity of PET in HCC diagnosis

<table>
<thead>
<tr>
<th>Authors</th>
<th>Journal</th>
<th>Year</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jeng, et al.</td>
<td>Hepatogastroenterology</td>
<td>2003</td>
<td>56%</td>
</tr>
<tr>
<td>Wudel, et al.</td>
<td>Am Surg</td>
<td>2003</td>
<td>64%</td>
</tr>
<tr>
<td>Ho, et al.</td>
<td>J Nucl Med</td>
<td>2003</td>
<td>47%</td>
</tr>
<tr>
<td>Khan, et al.</td>
<td>J Hepatol</td>
<td>2000</td>
<td>55%</td>
</tr>
</tbody>
</table>
PET in primary liver cancer

5. Higashi T, Hatano E, et al. FDG-PET as a prognostic predictor in the early post-therapeutic evaluation for unresectable hepatocellular carcinoma *Eur J Nucl Med Imaging* 2010
Predection of recurrence and prognosis after surgery by PET


Value of Fluorine-18-FDG-PET to Monitor Hepatocellular Carcinoma After Intervventional Therapy

Tatsuo Torizuka, Nagara Tamaki, Tetsuro Inokuma, Yasuhiro Magata, Yoshiharu Yonekura, Akira Tanaka, Yoshio Yamaoka, Kazutaka Yamamoto and Junji Konishi

Department of Nuclear Medicine and Second Department of Surgery, Kyoto University Faculty of Medicine, Kyoto; and Department of Radiology, Fukui Medical School, Fukui, Japan

Methods: Thirty-two tumors in 30 patients with hepatocellular carcinoma (HCC) were studied preoperatively using PET with $^{18}$F-labeled 2-fluoro-2-deoxy-D-glucose (FDG) to evaluate the metabolic activity of the lesions after interventional therapy. All patients had received transcatheter arterial chemoembolization therapy using iodized oil (Lipiodol, Laboratoire Guerbet, Aulnaysous-Bois, France) before the PET study. The tumors were 2 to 18 cm in diameter. FDG uptake at 48 to 60 min after tracer injection was used to determine the standardized uptake value (SUV). The SUVs of the tumor and nontumor regions of the liver were calculated to obtain the tumor-to-nontumor ratio (SUV ratio). The PET results were compared with the findings of CT and histologic examination. Results: The tumors were divided into three types, consisting of those with increased FDG uptake (SUV ratio of 1.07–2.66, Type A, n = 19), similar FDG uptake to the surrounding nontumor region (SUV ratio of 0.77–1.04, Type B, n = 7) and decreased or absent FDG uptake (SUV ratio of 0.13–0.58, Type C, n = 6). In histologic examination, viable HCC tissue remained in all Type A and B tumors, whereas more than half of Type C tumors, indicating necrosis. These PET findings reflect the extent of necrosis. Conclusion: FDG-PET is useful for the assessment of tumor viability in patients with HCC. To evaluate the metabolic activity of HCC after interventional therapy, preoperative FDG-PET was performed in patients with HCC, and the results were compared with CT and histologic findings.

MATERIALS AND METHODS

Subjects

Thirty preoperative patients with HCC (27 men and 3 women) with 32 tumors were studied. The patients ranged in age from 36 to 78 yr (average 59 yr). They had received interventional therapy 3 to 45 days (mean 26) before the PET study. For 20 tumors, transcatheter arterial infusion (TAI) was performed using 3 to 5 ml of iodized oil (Lipiodol Ultra-Fluide, Laboratoire Guerbet, Aulnay-sous-Bois, France) mixed with anticancer drugs. For 11 tumors, transcatheter arterial embolization (TAE) was performed.

Group A; TNR↑
Group B; TNR →
Group C; TNR ↓

90% necrosis in Group C

1994
Overall survival depending on TNR

Overall survival (%)

Days after surgery

TNR < 2

TNR > 2

MST 2310 days

MST 182 days

P < 0.01

Enrollment Flow

Prospective study

Admission (2003-2005)

188 pts

Candidates for curative surgery

140 pts

CT and MRI

93 pts

Informed consent

2 pts; Distant metastasis
21 pts; previous treatment
(18 TACE, 3 RFA)

70 pts

FDG-PET

Entry

FDG-PET predicts outcome after resection in HCC

Recurrence free survival

- Low SUV<5.0, n=40
- High SUV>5.0, n=30

\[ p = 0.0005 \]

- Low TNR<2.0, n=48
- High TNR>2.0, n=22

\[ p = 0.0002 \]
FDG-PET predicts outcome after resection in HCC

Overall survival

- Low SUV<5.0, n=40
- High SUV>5.0, n=30
  \[ p = 0.002 \]

- Low TNR<2.0, n=48
- High TNR>2.0, n=22
  \[ p = 0.0001 \]
Multivariate analysis of prognostic factors for disease-free and overall survival

<table>
<thead>
<tr>
<th>Disease-free survival</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP level</td>
<td>1.82-16.4</td>
<td>0.003</td>
</tr>
<tr>
<td>Maximal tumor dimension</td>
<td>0.99-1.02</td>
<td>0.31</td>
</tr>
<tr>
<td>Histological grade</td>
<td>0.3-2.14</td>
<td>0.65</td>
</tr>
<tr>
<td>Portal vein thrombus</td>
<td>1.49-14.5</td>
<td>0.008</td>
</tr>
<tr>
<td>SUV</td>
<td>0.17-2.5</td>
<td>0.54</td>
</tr>
<tr>
<td>TNR</td>
<td>1.03-1.62</td>
<td>0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Overall survival</th>
<th></th>
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<tbody>
<tr>
<td>Age</td>
<td>0.97-1.11</td>
<td>0.32</td>
</tr>
<tr>
<td>AFP level</td>
<td>1.89-40.9</td>
<td>0.006</td>
</tr>
<tr>
<td>Histological grade</td>
<td>0.37-6.2</td>
<td>0.56</td>
</tr>
<tr>
<td>Portal vein thrombus</td>
<td>0.37-3.83</td>
<td>0.76</td>
</tr>
<tr>
<td>SUV</td>
<td>0.34-8.24</td>
<td>0.52</td>
</tr>
<tr>
<td>TNR</td>
<td>1.07-2.38</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Prediction of treatment response by PET

Higashi T, Hatano E, et al. FDG-PET as a prognostic predictor in the early post-therapeutic evaluation for unresectable hepatocellular carcinoma

*Eur J Nucl Med Imaging* 2010
Evaluation with PET after HAIC

Hard to measure due to unclear margin of the tumors
Evaluation with PET after HAIC

SUV=8.80, TNR=2.79

SUV=4.61, TNR=1.72

HAIC (FP)
Evaluation with PET after HAIC

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUV</td>
<td>13.2</td>
<td>8.29</td>
</tr>
<tr>
<td>TNR</td>
<td>7.4</td>
<td>2.52</td>
</tr>
<tr>
<td>AFP</td>
<td>557310</td>
<td>166802</td>
</tr>
<tr>
<td>PIVKA II</td>
<td>6400</td>
<td>92</td>
</tr>
</tbody>
</table>
FDG PET as a prognostic predictor in the early post-therapeutic evaluation for unresectable hepatocellular carcinoma

Tatsuya Higashi · Etsuro Hatano · Iwao Ikai · Ryuichi Nishii · Yuji Nakamoto · Koichi Ishizu · Tsuyoshi Suga · Hidekazu Kawashima · Kaori Togashi · Satoru Seo · Koji Kitamura · Yasuji Takada · Shinji Kamimoto

Evaluation within 1 months after non-operative treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>TACE</td>
<td>24</td>
</tr>
<tr>
<td>HAI</td>
<td>31</td>
</tr>
<tr>
<td>RFA</td>
<td>5</td>
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<tr>
<td>Systemic chemotherapy</td>
<td>7</td>
</tr>
</tbody>
</table>
Survival Curve of HCC patients by Kaplan-Meier analysis

Log-rank test $P<0.0001$

Low FDG group:  
n=26, average 607.9+/−29.7

High FDG group:  
n=32, average 327.5+/-40.1
Survival Curve of HCC patients by Kaplan-Meier analysis--patients classified by FDG uptake pattern--

Log-rank test $P<0.01$
Prediction of differentiation by PET

Well differentiated HCC
55 yo, female
Moderately differentiated HCC

67 yo, female
Poorly differentiated HCC
63 yo, male
FDG-PET predicts tumor differentiation

What determines the FDG uptake in HCC?
Mechanism of FDG uptake in HCC

Uptake of FDG to Cancer Cells

18F-FDG

Membrane

18F-FDG

Hexokinase

18F-FDG-6-phosphate

Glucose-6-phosphatase

Glucose-6-phosphate

Glucose

Via Glucose Transporter

Metabolic trapping
Accumulation of 18F-FDG as glucose analogue

Glycolysis
Glucose metabolism in HCCs

<table>
<thead>
<tr>
<th></th>
<th>GLUT-1</th>
<th>GLUT-2</th>
<th>HK-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>well</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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<tr>
<td>mod</td>
<td><img src="image4.png" alt="Image" /></td>
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<tr>
<td>por</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Tumor differentiation is correlated with GLUT-1

GLUT-1

* P = 0.043

Tumor differentiation

HK-II

G-6-Pase

Tumor differentiation

well
n=8
mod
n=14
por
n=6

well
n=8
mod
n=14
por
n=6

well
n=5
mod
n=12
por
n=3
SUV is correlated with GLUT-1

- GLUT-1: $p < 0.0001$, $r = 0.74$
- GLUT-2: $p = 0.83$, $r = -0.04$
- HK-II: $p = 0.4$, $r = 0.17$
- G-6-Pase: $p = 0.2$, $r = -0.31$
TNR is correlated with GLUT-1

GLUT-1

$p < 0.0001$

$r = 0.69$

GLUT-2

$p = 0.89$

$r = -0.03$

HK-II

$p = 0.4$

$r = 0.17$

G-6-Pase

$p = 0.3$

$r = -0.27$
Relationship between glucose metabolism and FDG uptake in HCCs

- FDG uptake was not associated with the expression of GLUT-2 and HK-II or G-6-Pase activity.
- FDG uptake was correlated with GLUT-1 expression, though the level of the expression was relatively weak.
Seo S, Hatano E, et al.
P-glycoprotein expression affects $^{18}$F- fluorodeoxyglucose accumulation in hepatocellular carcinoma \textit{in vivo} and \textit{in vitro}.

\textit{Int J Oncol} 2009
P-glycoprotein

- Multidrug resistance (MDR)-induced protein
  - P-glycoptein
  - Multidrug resistance-associated protein (MRP)
  - Breast cancer-related protein (BCRP)

- P-gp expression inversely correlates with chemotherapeutic response to etoposide or doxorubicin in HCC
P-gp expression (%)

Tumor differentiation

* $P = 0.0001$
† $P < 0.0001$

well (n=9)
mod (n=14)
poor (n=6)
FDG-PET predicts P-glycoprotein expression in HCC

Inverse correlation between P-gp expression and SUV (TNR)
The role of P-gp in FDG uptake in vitro

Cells

- human epidermoid carcinoma cell line
  - KB 3-1 : P-gp(-)
  - KB V-1 : P-gp(+)

- human HCC cell line
  - PLC/PRF/5 : P-gp(-)
  - PLC/DOR : P-gp(+)

Inhibitors of P-gp

- verapamil (10μM)
- cepharanthine (5μM)
In vitro character of HCC cell line

<table>
<thead>
<tr>
<th>Protein</th>
<th>KB V-1</th>
<th>KB 3-1</th>
<th>PLC/DOR</th>
<th>PLC/PRF/5</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-gp</td>
<td></td>
<td></td>
<td></td>
<td>170 KDa</td>
</tr>
<tr>
<td>GLUT-1</td>
<td></td>
<td></td>
<td></td>
<td>65 KDa</td>
</tr>
<tr>
<td>GLUT-2</td>
<td></td>
<td></td>
<td></td>
<td>55 KDa</td>
</tr>
<tr>
<td>HK-Ⅱ</td>
<td></td>
<td></td>
<td></td>
<td>50 KDa</td>
</tr>
<tr>
<td>β-actin</td>
<td></td>
<td></td>
<td></td>
<td>102 KDa</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>43 KDa</td>
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</table>
P-gp expression attenuated FDG uptake

KB cell line

HCC cell line
Verapamil increased FDG uptake in P-gp(+) cells

KB cell line

HCC cell line
Cepharanthin increased FDG uptake in P-gp(+) cells

For KB cell line:
- KB V-1: Blue line
- KB V-1 + Cepharanthin: Red line

For HCC cell line:
- PLC/DOR: Blue line
- PLC/DOR + Cepharanthin: Red line

- Test for KB cell line: $p = 0.002$
- Test for HCC cell line: $p = 0.0001$
Is FDG a substrate of P-gp?

- FDG uptake in P-gp (-) cells was higher than that in P-gp (+) cells.

- Inhibitors of P-gp increased FDG uptake in P-gp (+) cells.

(Seo S et al. *Int J Oncol*, 2009)
Can FDG-PET predict chemo-sensitivity in HCC?

• Patients
  – 15 HCC pts underwent HAIC (FP)

• Groups; High SUV>6.0 vs. Low SUV<6.0
• Groups; High TNR>2.5 vs. Low TNR<2.5
Prediction of proliferative activity by PET

Kitamura K, Hatano E, et al.
Proliferative activity in hepatocellular carcinoma is closely correlated with glucose metabolism but not angiogenesis.
J Hepatology 2011
Assessment for Malignant potential, Angiogenesis and Glucose metabolism

- **Malignant potential**
  - Ki-67 LI (proliferative activity) • • • IHC

- **Angiogenesis**
  - VEGF • • • • • • IHC
  - Western blot (WB) analysis
  - MVD; microvessel density (CD34) • • • • • IHC

- **Glucose metabolism**
  - GLUT-1 • • • • • • IHC, WB analysis
  - FDG - PET • • • • • • SUV, TNR
  - PK-M2
63 patients were divided into 3 groups according to Ki-67 labeling index (LI)

- < 10% low LI: L group (n = 30)
- 10% - 30% intermediate LI: I group (n = 20)
- > 30% high LI: H group (n = 13)
Ki-67 LI was significantly associated with prognosis in 63 patients.

- Low LI (L群, n = 30): < 10%
- Intermediate LI (I群, n = 20): 10% - 30%
- High LI (H群, n = 13): > 30%

Cumulative survival rate and cumulative recurrence free survival rate showed significant differences (P < 0.001) among the three groups.
Immunostaining for VEGF

Percentage of stained tumor cells

<table>
<thead>
<tr>
<th>Staining intensity</th>
<th>1 – 25%</th>
<th>26 – 50%</th>
<th>51 – 75%</th>
<th>76 – 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Moderate</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Strong</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

VEGF expression was inversely correlated with Ki-67 LI.
VEGF western blot analysis

**Graph:**
- **NTL**, **L群**, **I群**, **H群**
- **VEGF** and **Actin** bands
- **p < 0.05**
- **p < 0.01**

**Bar graph:**
- **VEGF/Actin (ratio to control)**
- **6**, **21**, **16**, **9**
- **n**
Evaluation of MVD by IHC for CD34

10 randomly chosen high-power fields from each specimen were evaluated to calculate the positively stained areas using NIH image.

\[
\text{CD34 expression (\%)} = \frac{\text{average pixel number of CD34 positive area}}{\text{pixel number of whole area}} \times 200
\]

MVD (CD34) was reduced according to Ki-67 LI

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>L群</td>
<td>11.6 ± 5.6</td>
<td>30</td>
</tr>
<tr>
<td>I群</td>
<td>7.6 ± 3.7</td>
<td>20</td>
</tr>
<tr>
<td>H群</td>
<td>3.3 ± 3.7</td>
<td>13</td>
</tr>
</tbody>
</table>
MVD (CD34) was correlated with VEGF expression

\[ r = 0.790 \]
Immunostaining for GLUT-1

0 = 0%
1 = 1% - 25%
2 = 26% - 50%
3 = 51% - 75%
4 = 76% - 100%

GLUT-1 expression was enhanced in High Ki-67 LI
GLUT-1 — western blot analysis

GLUT-1

Actin

NTL  L群  I群  H群

GLUT-1/Actin (ratio to control)

0.0  0.2  0.4  0.6

6  21  16  9  (n)

p < 0.001

p < 0.01
PK-M2

✓ Pyruvate kinase
✓ Final step in glycolysis
✓ phosphoenolpyruvate → pyruvate + ATP

[Christofk et al. Nature 2008]

LETTERS

The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth

Heather R. Christofk³, Matthew G. Vander Heiden¹,², Marian H. Harris³, Arvind Ramanathan⁴, Robert E. Gerszten⁵,⁶, Ru Wei⁷, Mark D. Fleming³, Stuart L. Schreiber⁴,⁷ & Lewis C. Cantley¹,⁸

[Christofk et al. Nature 2008]

✓ ---- increase both in activity and amount of PKM2 in hepatocellular tumors.
   [K Tani et al. Gene 1988]

✓ PKM gene expression was significantly higher in low-differentiated HCC than in well-differentiated HCC.
   [T Hamaguchi et al. I.J of oncology 2008]
glucose transporters

Hexokinase (HK II)

glycolysis

phosphoenolpyruvate

PK(M2)

pyruvate

HIF1α

LDH5

lactate

anaerobic glycolysis

hypoxia

PDK1

PDH

Acetyl-coA

TCA cycle

anaerobic glycolysis

aerobic glycolysis
Correlation between PKM2 and FDG uptake

**SUV < 5**
- PKM2/β-actin: 0.8 ± 0.1
- n = 24

**SUV ≥ 5**
- PKM2/β-actin: 2.0 ± 0.3
- n = 22

**TNR < 2**
- PKM2/β-actin: 0.9 ± 0.1
- n = 32

**TNR ≥ 2**
- PKM2/β-actin: 2.5 ± 0.5
- n = 14

*P < 0.01

**P < 0.05**

**r = 0.528**

**r = 0.343**

*P < 0.01
Correlation between PKM2 and Ki-67 LI

- **L 群**: 0.9 ± 0.1
  - n = 21

- **I 群**: 1.5 ± 0.3
  - n = 16

- **H 群**: 2.3 ± 0.6
  - n = 9

- **P** < 0.01
- **r** = 0.386

The graph shows a scatter plot with a linear regression line indicating a positive correlation between PKM2/β-actin and Ki-67 LI. The correlation coefficient (r) is 0.386, and the correlation is statistically significant (P < 0.01).
SUV and TNR were enhanced according to Ki-67 LI

**SUV**

- L群 (n=30): 3.6 ± 1.4
- L群 (n=20): 5.7 ± 3.0
- H群 (n=13): 11.4 ± 6.9

**TNR**

- L群 (n=30): 1.3 ± 0.5
- L群 (n=20): 2.0 ± 1.0
- H群 (n=13): 4.5 ± 3.2
GLUT-1 expression was located in the area separated from tumor vessels. GLUT-1 was mainly expressed in a relatively-hypoxic area away from the tumor vessels.
Malignant potential in advanced HCC closely correlate with glucose metabolism but not angiogenesis.
Prediction of recurrence patterns by PET

Kitamura K, Hatano E, et al.
Preoperative FDG-PET Predicts Recurrence Patterns in Hepatocellular Carcinoma.
Cumulative survival rates and TNR according to recurrence patterns.
Cumulative survival rates and TNR according to time interval before recurrence.
## Multivariate analysis of predictive factors

(Logistic regression analysis)

### for recurrence patterns

<table>
<thead>
<tr>
<th></th>
<th>Relative risk</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIVKA II (400)</td>
<td>0.345</td>
<td>0.102 – 1.165</td>
<td>0.086</td>
</tr>
<tr>
<td>TNR (2)</td>
<td>0.262</td>
<td>0.080 – 0.850</td>
<td>0.026</td>
</tr>
</tbody>
</table>

### for interval before initial recurrence

<table>
<thead>
<tr>
<th></th>
<th>Relative risk</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (65)</td>
<td>2.766</td>
<td>0.725 – 10.552</td>
<td>0.136</td>
</tr>
<tr>
<td>T Stage (UICC)</td>
<td>1.428</td>
<td>0.260 – 7.837</td>
<td>0.682</td>
</tr>
<tr>
<td>AFP (400)</td>
<td>0.227</td>
<td>0.032 – 1.582</td>
<td>0.134</td>
</tr>
<tr>
<td>PIVKA II (400)</td>
<td>0.553</td>
<td>0.146 – 2.090</td>
<td>0.383</td>
</tr>
<tr>
<td>TNR (2)</td>
<td>0.164</td>
<td>0.038 – 0.716</td>
<td>0.016</td>
</tr>
</tbody>
</table>
Proposal

• HCC patients
  – with a TNR of $<2$ are candidates for LR as an initial surgical strategy.
  – with a TNR of $\geq 2$ are candidates for OLT or need adjuvant therapy.
Patient selection for liver transplantation by PET
The role of $^{18}$F-FDG-PET imaging for the selection of liver transplantation candidates among hepatocellular carcinoma patients
SH Yang et al. Liver Transplant 12 (11); 1655, 2006

Cumulative recurrence-free survival curve according to $^{18}$F-FDG-PET imaging.
Recurrence after liver transplantation

Moderately differentiated HCC
SUV=4.1, TNR=1.52
Six months after LDLT, bone metastasis was detected by CT.
No recurrence after liver transplantation in PET-negative HCC patients

Prospective study
22 LDLTs including 12 pts beyond Milano

<table>
<thead>
<tr>
<th>TNR</th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1.5</td>
<td>2*</td>
<td>3</td>
</tr>
<tr>
<td>&lt;1.5</td>
<td>0</td>
<td>17</td>
</tr>
</tbody>
</table>

* Beyond Milano
Prospective study

Kyoto Criteria

Since Jan 2007

- ≤5 cm
- N ≤10
- DCP ≤400
Patient survival rate

Overall survival

Years after LT

After Kyoto criteria

1-y 3-y
86% 80%
Recurrence rate

It is hard to show the usefulness of PET for selection in our cohort.
Conclusion

• The proliferative activity was closely correlated with the glucose metabolism estimated by PET, but not with angiogenesis.

• Preoperative FDG-PET predicts HCC recurrences within the MC or no recurrence and recurrences after 1 year or later.

• FDG-PET may be useful for assessing the malignant potential of HCC and selecting appropriate patients for liver resection but not for liver transplantation as an initial surgical strategy.
PET-based imaging is not accurate to stage early tumors. Pre-operative staging prior to liver transplantation should include abdominal dynamic CT or MRI, chest CT and bone scintigraphy.
Evaluation with PET after RFA

Percutaneous RFA x 3
Transthoracic RFA

SUV=12.7, TNR=5.15
A, analysis of SUVmax according to the status of KRAS and BRAF. SUVmax was significantly higher in patients with mutated KRAS or BRAF than in those with wild-type KRAS and BRAF (P = 0.006; exact Mann–Whitney U test).

Mutated KRAS increase $^{18}$F-FDG accumulation in CRC cells mainly by up-regulating the expression of GLUT1.

**Quantitative RT-PCR**

<table>
<thead>
<tr>
<th>KRAS MT</th>
<th>KRAS WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT116</td>
<td>HKh-2</td>
</tr>
<tr>
<td>HKe-3</td>
<td>HCT116</td>
</tr>
</tbody>
</table>

Relative GLUT1 mRNA expression

**Western blot**

<table>
<thead>
<tr>
<th>KRAS</th>
<th>GLUT1</th>
<th>HK2</th>
<th>ACTB</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**in vitro $^{18}$F-FDG accumulation**

- HCT116 (KRAS MT)
- HKh-2 (KRAS WT)
- HKe-3 (KRAS WT)

In vitro $^{18}$F-FDG uptake (%ID/mg)

- siCtrl
- siKRAS #1
- siKRAS #2
- siGLUT1 #1
- siGLUT1 #2
- siHK2 #1
- siHK2 #2

* P<0.01
† P<0.05
Xenograft tumors with mutated *KRAS* increase $^{18}$F-FDG accumulation and hypoxic lesion might affect on FDG accumulation through HIF-1α expression.
Hypoxia additively increase $^{18}$F-FDG accumulation in CRC cells with mutated KRAS at least partially through HIF-1$\alpha$ induction.

**[Western blot]**

**[in vitro FDG uptake]**

**[in vitro FDG uptake]**

**[Western blot]**

HCT116 (KRAS MT)

<table>
<thead>
<tr>
<th>siRNA</th>
<th>HIF-1$\alpha$</th>
<th>GLUT1</th>
<th>HK2</th>
<th>ACTB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>#1</td>
<td>#2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIF-1$\alpha$</td>
<td>Ctrl #1 #2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HCT116: Normoxia

- HCT116
- HKe-3

HCT116: Hypoxia

- HCT116
- HKe-3

$\ast$ P<0.01
51 clinical samples with primary CRC tumors; *KRAS* mutational status is correlated with FDG accumulation and expression of GLUT1 and HIF-1α.

**Representative cases**

**Case 1** *(KRAS MT)*

**Case 2** *(KRAS WT)*

<table>
<thead>
<tr>
<th><strong>KRAS status</strong></th>
<th>MT (n=22)</th>
<th>WT (n=29)</th>
<th><em>P</em> value</th>
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<tbody>
<tr>
<td><strong>GLUT1 expression</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- or +</td>
<td>10</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>12</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>HK2 expression</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- or +</td>
<td>8</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>14</td>
<td>18</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>HIF-1α expression</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>10</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>12</td>
<td>7</td>
<td>0.026</td>
</tr>
<tr>
<td><strong>SUVmax</strong></td>
<td>Mean ± SD</td>
<td>16.7 ± 6.6</td>
<td>12.8 ± 6.6</td>
</tr>
</tbody>
</table>

Relationship between *KRAS* mutational status and expression of GLUT1, HK2, HIF-1α and FDG accumulation.
The sensitivity of F-18 FDG PET in HCC patients is 45 - 65%

C-11 Acetate has high sensitivity (87.3%) in HCC

Half-life of C-11 Acetate is too short (20.4 min) to use as commercial base

F-18 Fluoroacetate (FACE)
Metabolic Pathway of Fluoroacetate

Glucose transport

Glucose

Fluoroacetate

F-AcCoA

Fluorocitrate

Citrate → Cisaconitate

Isocitrate

α-Oxoglutarate

Succinate

Oxaloacetate

L-Malate

Fumarate

Acetate

mitochondrion

TCA Cycle

Aconitase binding / inhibit
Healthy Volunteer Study
Patient Study

81 y.o., male, well-differentiated HCC

67 y.o., male, unknown HCC
## Characteristics of ten patients with liver tumor

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Sex</th>
<th>Tumor</th>
<th>Differentiation of HCC</th>
<th>Tumor size (mm)</th>
<th>FACE uptake of Tumor</th>
<th>FDG uptake of Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SUVmax</td>
<td>TNR</td>
</tr>
<tr>
<td>1</td>
<td>81</td>
<td>M</td>
<td>HCC</td>
<td>Well</td>
<td>10</td>
<td>FN</td>
<td>2.18</td>
</tr>
<tr>
<td>2</td>
<td>77</td>
<td>F</td>
<td>HCC</td>
<td>Well/Moderate</td>
<td>130</td>
<td>TP</td>
<td>3.37</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>M</td>
<td>HCC</td>
<td>Moderate</td>
<td>17</td>
<td>TP</td>
<td>3.04</td>
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<tr>
<td>4</td>
<td>65</td>
<td>M</td>
<td>HCC</td>
<td>Unknown</td>
<td>23</td>
<td>FN</td>
<td>2.30</td>
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<td>5</td>
<td>67</td>
<td>M</td>
<td>HCC</td>
<td>Unknown</td>
<td>40</td>
<td>TP</td>
<td>3.62</td>
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<tr>
<td>6</td>
<td>74</td>
<td>F</td>
<td>CCC</td>
<td></td>
<td>107</td>
<td>TP</td>
<td>3.05</td>
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<tr>
<td>7</td>
<td>70</td>
<td>M</td>
<td>Metastatic Colon Cancer</td>
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<td>104</td>
<td>TP</td>
<td>2.74</td>
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<td>8</td>
<td>75</td>
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<td>–</td>
<td>–</td>
<td>Not performed</td>
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<tr>
<td>9</td>
<td>78</td>
<td>M</td>
<td>Metastatic Colon Cancer</td>
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<tr>
<td>10</td>
<td>58</td>
<td>F</td>
<td>Metastatic Pancreatic Endocrine Tumor</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tbody>
</table>

**Terms**
- **TN**: the metastatic nodule disappeared shortly after FACE PET/CT study
- **FN**: negative finding at early scan (60 minutes), but faint uptake was observed at delayed scan (120 minutes)
Expression of KL-6, a type of sialylated carbohydrate antigen which is located on MUC1 mucin, has been reported to be associated with prognosis in various types of gastrointestinal cancers. However the relationship between KL-6 and prognosis in patients with hepatocellular carcinoma (HCC) has not been reported so far. The aim of this study is to evaluate the relationship between the expressions of KL-6 and the clinicopathological factors including standardized uptake value with FDG-PET in HCC patients.
Representative image of CT, FDG-PET & immunohistochemical figure for KL-6
Univariate analysis of preoperative clinical factors associated with KL-6 expression

<table>
<thead>
<tr>
<th>Features</th>
<th>n</th>
<th>KL-6 expression</th>
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<th>P value</th>
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<td></td>
<td></td>
<td></td>
<td>Negative (%)</td>
<td>Positive (%)</td>
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<tr>
<td>All cases</td>
<td>61</td>
<td>56</td>
<td>(91.8)</td>
<td>5</td>
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<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45</td>
<td>42</td>
<td>(93.3)</td>
<td>3</td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>14</td>
<td>(87.5)</td>
<td>2</td>
</tr>
<tr>
<td>Age</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>&lt;65</td>
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<td>24</td>
<td>(88.9)</td>
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<tr>
<td>≥65</td>
<td>34</td>
<td>32</td>
<td>(94.1)</td>
<td>2</td>
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<tr>
<td>Preoperative AFP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;400</td>
<td>48</td>
<td>45</td>
<td>(93.8)</td>
<td>3</td>
</tr>
<tr>
<td>≥400</td>
<td>13</td>
<td>11</td>
<td>(84.6)</td>
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<tr>
<td>Preoperative PIVKA-II</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;400</td>
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<td>20</td>
<td>(87.0)</td>
<td>3</td>
</tr>
<tr>
<td>≥400</td>
<td>38</td>
<td>36</td>
<td>(94.7)</td>
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<tr>
<td>Child –Pugh score</td>
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</tr>
<tr>
<td>A</td>
<td>57</td>
<td>52</td>
<td>(91.2)</td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>4</td>
<td>(100.0)</td>
<td>0</td>
</tr>
<tr>
<td>TNM Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>29</td>
<td>27</td>
<td>(93.1)</td>
<td>2</td>
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<tr>
<td>II–III</td>
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<td>Differentiation</td>
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<td></td>
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<tr>
<td>Well/Mod</td>
<td>44</td>
<td>43</td>
<td>(97.7)</td>
<td>1</td>
</tr>
<tr>
<td>Por</td>
<td>17</td>
<td>13</td>
<td>(76.5)</td>
<td>4</td>
</tr>
<tr>
<td>TNR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2</td>
<td>42</td>
<td>41</td>
<td>(97.6)</td>
<td>1</td>
</tr>
<tr>
<td>≥2</td>
<td>19</td>
<td>15</td>
<td>(79.0)</td>
<td>4</td>
</tr>
<tr>
<td>GLUT-1 expression</td>
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<td></td>
</tr>
<tr>
<td>Negative</td>
<td>38</td>
<td>38</td>
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<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>23</td>
<td>18</td>
<td>(78.3)</td>
<td>5</td>
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</table>
## Univariate & multivariate analysis of prognostic factors for disease-free & overall survival

<table>
<thead>
<tr>
<th>Variables (Disease free survival)</th>
<th>Univariate</th>
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<th></th>
<th>Multivariate</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>CI (95%)</td>
<td>P value</td>
<td>HR</td>
<td>CI (95%)</td>
<td>P value</td>
</tr>
<tr>
<td>KL-6 expression</td>
<td>5.321</td>
<td>1.751–13.325</td>
<td>0.0054 *</td>
<td>3.807</td>
<td>1.092–11.798</td>
<td>0.0370 *</td>
</tr>
<tr>
<td>Sex</td>
<td>1.156</td>
<td>0.586–2.145</td>
<td>0.6624</td>
<td>1.145</td>
<td>0.535–2.352</td>
<td>0.7184</td>
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<tr>
<td>Age</td>
<td>0.661</td>
<td>0.372–1.180</td>
<td>0.1597</td>
<td>1.388</td>
<td>0.641–3.065</td>
<td>0.4071</td>
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<tr>
<td>Preoperative AFP</td>
<td>2.622</td>
<td>1.294–4.960</td>
<td>0.0089 *</td>
<td>2.394</td>
<td>0.903–6.186</td>
<td>0.0783</td>
</tr>
<tr>
<td>Preoperative PIVKA–II</td>
<td>1.674</td>
<td>0.921–3.186</td>
<td>0.0922</td>
<td>1.177</td>
<td>0.533–2.646</td>
<td>0.6878</td>
</tr>
<tr>
<td>Child –Pugh score</td>
<td>3.128</td>
<td>0.927–7.950</td>
<td>0.0636</td>
<td>4.621</td>
<td>1.089–16.432</td>
<td>0.0390 *</td>
</tr>
<tr>
<td>TNM Stage</td>
<td>2.238</td>
<td>1.248–4.081</td>
<td>0.0069 *</td>
<td>1.735</td>
<td>0.825–3.728</td>
<td>0.147</td>
</tr>
<tr>
<td>Differentiation</td>
<td>3.600</td>
<td>1.880–6.643</td>
<td>0.0002 *</td>
<td>3.197</td>
<td>1.293–7.837</td>
<td>0.0123 *</td>
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<tr>
<td>TNR</td>
<td>3.057</td>
<td>1.646–5.531</td>
<td>0.0006 *</td>
<td>1.121</td>
<td>0.486–2.604</td>
<td>0.7873</td>
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<tr>
<td>GLUT-1 expression</td>
<td>2.712</td>
<td>1.499–4.852</td>
<td>0.0012 *</td>
<td>1.418</td>
<td>0.605–3.404</td>
<td>0.4227</td>
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</table>

<table>
<thead>
<tr>
<th>Variables (Overall survival)</th>
<th>Univariate</th>
<th></th>
<th></th>
<th>Multivariate</th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>HR</td>
<td>CI (95%)</td>
<td>P value</td>
<td>HR</td>
<td>CI (95%)</td>
<td>P value</td>
</tr>
<tr>
<td>KL-6 expression</td>
<td>5.686</td>
<td>1.868–14.329</td>
<td>0.0041 *</td>
<td>3.937</td>
<td>1.093–13.485</td>
<td>0.0370 *</td>
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<tr>
<td>Sex</td>
<td>0.963</td>
<td>0.379–2.162</td>
<td>0.9314</td>
<td>0.679</td>
<td>0.230–1.800</td>
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<tr>
<td>Age</td>
<td>0.700</td>
<td>0.331–1.483</td>
<td>0.3481</td>
<td>0.807</td>
<td>0.295–2.170</td>
<td>0.6702</td>
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<td>Preoperative AFP</td>
<td>2.709</td>
<td>1.160–5.871</td>
<td>0.0229</td>
<td>1.525</td>
<td>0.465–4.587</td>
<td>0.4718</td>
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<td>Preoperative PIVKA–II</td>
<td>1.590</td>
<td>0.725–3.843</td>
<td>0.2546</td>
<td>0.597</td>
<td>0.209–1.747</td>
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<td>Child –Pugh score</td>
<td>2.992</td>
<td>0.704–8.755</td>
<td>0.1221</td>
<td>3.098</td>
<td>0.533–14.306</td>
<td>0.1918</td>
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<td>2.858</td>
<td>1.304–6.903</td>
<td>0.0080 *</td>
<td>2.793</td>
<td>1.009–8.701</td>
<td>0.0479 *</td>
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<tr>
<td>Differentiation</td>
<td>1.92</td>
<td>0.851–4.092</td>
<td>0.1123</td>
<td>0.616</td>
<td>0.220–1.646</td>
<td>0.3369</td>
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<tr>
<td>TNR</td>
<td>4.35</td>
<td>2.048–9.362</td>
<td>0.0002 *</td>
<td>1.876</td>
<td>0.616–5.770</td>
<td>0.2658</td>
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<tr>
<td>GLUT-1 expression</td>
<td>4.35</td>
<td>2.043–9.649</td>
<td>0.0002 *</td>
<td>3.466</td>
<td>1.169–10.770</td>
<td>0.0248 *</td>
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</table>
Survival curve of HCC patients by Kaplan-Meier analysis

KL-6 expression predicts survival after resection in patients with HCC, indicating KL-6 might be an important biomarker as well as TNR in FDG-PET.