**Supplementary Data**

**Legend for Supplementary Figure**

**Supplementary Figure 1.** Example of combined immunofluorescent staining and a three-color filter-adapted fluorescent in situ hybridization (FA-FISH) experiment using filtration enriched-H2228 cell line spiked into peripheral blood samples from a healthy donor and *anaplastic lymphoma kinase* (*ALK*) break-apart probes coupled to the chromosome 2-specific centromeric probe. (A) Example of a white blood cell (WBC). (B) Example of a H2228 cell. Scale: bars = 10 µm.

**Supplementary Table 1.** Detection of *anaplastic lymphoma kinase* (*ALK*)-Rearrangement and *ALK* copy number gain in Tumors and Circulating Tumor Cells of *ALK*-Rearranged Patients

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  | **Tumor** a | |  | **CTCs** | | | | | | | | | | | | | | | |
|  |  |  |  |  | |  | **Characterized by ISET** b | | | | | | |  | **CellSearch** | | | | | | | |
|  |  |  |  |  |  |  | **Baseline** | |  | | **On crizotinib** | | |  | **Baseline** | | |  | | **On crizotinib** | | |
| **Patients** | **ORR** | **PFS**  **(months)** | **OS**  **(months)** | **% of *ALK*-Rearranged Cells** | **% of  *ALK*- CNG Cells** |  | ***ALK*-Rearranged CTCs**  **(/3 mL)** | ***ALK*-CNG CTCs**  **(/3 mL)** | |  | | ***ALK*-Rearranged CTCs**  **(/3 mL)** | ***ALK*-CNG CTCs**  **(/3 mL)** | | |  | **CTCs counts (/7.5 mL)** | |  | | **CTCs counts (/7.5 mL)** |
| **P1** | PR | 9.8 | 13.4 | 97 | 0 |  | 20 | 12 | |  | | - | - | | |  | 0 | |  | | - |
| **P2** | PR | 12.6 | 16.1 | 47 | 0 |  | 27 | 3 | |  | | - | - | | |  | 0 | |  | | - |
| **P3** | SD | 22.5 | 24.3 | 30 | 0 |  | 18 | 39 | |  | | 26 | 70 | | |  | 2 | |  | | 0 |
| **P4** | PD | 1.5 | 2.2 | 30 | 55 |  | 14 | 8 | |  | | 57 | 177 | | |  | 713 | |  | | 544 |
| **P5** | PR | 25.2 | 34.5 | 58 | 16 |  | 20 | 15 | |  | | 27 | 12 | | |  | 0 | |  | | 1 |
| **P6** | PR | 2.0 | 20.2 | 43 | 32 |  | 119 | 2 | |  | | 55 | 14 | | |  | 0 | |  | | 0 |
| **P7** | NA | 0.8 | 0.8 | 27 | 28 |  | 20 | 44 | |  | | - | - | | |  | 16 | |  | | - |
| **P8** | SD | 6.1 | 7.3 | RT-PCR+ c |  |  | 50 | 8 | |  | | 60 | 29 | | |  | 12 | |  | | 0 |
| **P9** | SD | 28.8 | 48.8\* | 61 | 0 |  | 13 | 23 | |  | | 3 | 19 | | |  | 0 | |  | | 0 |
| **P10** | NA | 10.4 | 29.1 | 31 | 0 |  | 15 | 9 | |  | | - | - | | |  | 6 | |  | | - |
| **P11** | SD | 3.4 | 14.3 | 68 | 0 |  | 14 | 13 | |  | | 23 | 34 | | |  | 0 | |  | | 0 |
| **P12** | PR | 9.8 | 49.5\* | 29 | 0 |  | 14 | 15 | |  | | 26 | 15 | | |  | 0 | |  | | 0 |
| **P13** | PR | 7.9 | 11.5 | 44 | 0 |  | 12 | 21 | |  | | 8 | 15 | | |  | 1 | |  | | 0 |
| **P14** | SD | 6.9 | 42.6\* | 62 | 8 |  | 21 | 10 | |  | | 20 | 35 | | |  | 0 | |  | | 0 |
| **P15** | PD | 1.0 | 4.0 | 25 | 13 |  | 11 | 1 | |  | | 21 | 26 | | |  | 5 | |  | | 94 |
| **P16** | PR | 18.3 | 45.2\* | 87 | 0 |  | 18 | 5 | |  | | 18 | 21 | | |  | 0 | |  | | 0 |
| **P17** | PR | 32.9 | 43.1\* | 25 | 1 |  | 14 | 5 | |  | | - | - | | |  | 0 | |  | | - |
| **P18** | SD | 14.0 | 16.2 | 27 | 4 |  | 40 | 6 | |  | | 9 | 2 | | |  | 0 | |  | | 4 |
| **P20** | PR | 39.6\* | 39.6\* | 77 | 0 |  | 13 | 6 | |  | | 11 | 2 | | |  | 0 | |  | | 1 |
| **P21** | PR | 39.2\* | 39.2\* | 31 | 53 |  | 17 | 14 | |  | | 6 | 4 | | |  | 1 | |  | | 0 |
| **P22** | SD | 2.5 | 2.7 | 25 | 17 |  | 4 | 11 | |  | | 41 | 58 | | |  | 0 | |  | | 0 |
| **P23** | PR | 5.8 | 33.2 | 4 d | 69 |  | 12 | 6 | |  | | 16 | 8 | | |  | 0 | |  | | 0 |
| **P24** | SD | 1.6 | 5.7 | 21 | 41 |  | 26 | 36 | |  | | 11 | 18 | | |  | 1 | |  | | 0 |
| **P25** | PD | 1.7 | 6.2 | 15 | 23 |  | 24 | 33 | |  | | 28 | 15 | | |  | 7 | |  | | 5 |
| **P26** | PR | 4.7 | 24.7 | 9 d | 16 |  | 11 | 21 | |  | | 13 | 12 | | |  | 0 | |  | | 0 |
| **P27** | PD | 1.7 | 3.0 | 19 | 30 |  | 13 | 31 | |  | | - | - | | |  | 5 | |  | | - |
| **P28** | PD | 0.8 | 4.0 | 12 d | 46 |  | 20 | 40 | |  | | - | - | | |  | N/A | |  | | - |
| **P29** | PD | 1.6 | 11.3 | 33 | 25 |  | 7 | 5 | |  | | 10 | 15 | | |  | 19 | |  | | 65 |
| **P30** | SD | 29.2\* | 29.2\* | IHC+ e |  |  | 31 | 53 | |  | | - | - | | |  | 1 | |  | | - |
| **P31** | SD | 1.4 | 3.3 | 46 | 32 |  | 11 | 13 | |  | | 6 | 36 | | |  | 0 | |  | | 0 |
| **P32** | PR | 6.7 | 16.8 | 62 | 18 |  | 15 | 3 | |  | | 12 | 12 | | |  | 5 | |  | | 0 |
| **P33** | PD | 2.3 | 16.3 | 26 | 32 |  | 10 | 15 | |  | | 4 | 6 | | |  | 2 | |  | | 0 |
| **P34** | PR | 25.0\* | 25.0\* | 34 | 6 |  | 4 | 10 | |  | | 5 | 2 | | |  | 0 | |  | | 0 |
| **P35** | SD | 9.7 | 26.3\* | 83 | 0 |  | 12 | 19 | |  | | 20 | 27 | | |  | 2 | |  | | 0 |
| **P36** | SD | 3.2 | 22.6\* | 76 | 15 |  | 8 | 7 | |  | | - | - | | |  | 0 | |  | | - |
| **P37** | PR | 8.1 | 17.3\* | 37 | 0 |  | 7 | 12 | |  | | 12 | 25 | | |  | 0 | |  | | 0 |
| **P38** | SD | 1.0 | 1.5 | 51 | 10 |  | 26 | 32 | |  | | - | - | | |  | 0 | |  | | - |
| **P39** | PR | 15.4\* | 15.4\* | 78 | 0 |  | 7 | 13 | |  | | 6 | 9 | | |  | 0 | |  | | 0 |
| **P40** | PD | 0.5 | 1.6 | 18 | 15 |  | 1 | 8 | |  | | 3 | 6 | | |  | 3 | |  | | 1 |

Abbreviations: *ALK*, anaplastic lymphoma kinase; CNG, copy number gain; CTC, circulating tumor cell; FA-FISH, filter-adapted FISH; FISH, fluorescence *in situ* hybridization; IF, immunofluorescent staining; IHC, immunohistochemistry; N/A, not available; ORR, overall response rate; PD, progression disease; PR, partial response; RT-PCR, reverse transcription polymerase chain reaction; SD, stable disease; \* censured patient.

a FISH results in the tumor biopsies. When useful, FISH was coupled to IHC or RT-PCR to access positivity.

b Three ISET spots were analyzed by combined IF and FA-FISH. Results are expressed as the numbers of *ALK*-rearranged CTC and CTC with gain of *ALK*-copies per 3 mL of blood.

c The biopsy was negative by FISH but positive by RT-PCR.

d Patient P23 had 4% of *ALK*-rearranged cells in the tumor biopsy which contained only 10% of tumor cells. In spite of this insufficient percentage the medical staff decided to treat the patient P23 with crizotinib because this patient had clinical characteristics of *ALK*-positive patients. The first tumor biopsy from P26 contained 9% of *ALK*-rearranged cells and the second biopsy was negative by IHC. Similarly, despite this insufficient percentage, the medical staff also decided to treat this patient with crizotinib based on clinical characteristics of *ALK*-positive patients. *ALK*-positivity was under the standard threshold for patient P28, however, the same medical decision was made for this patient bearing clinical characteristics of *ALK*-positive patients. For these three patients (P23, P26 and P28), *ALK* diagnosis was hindered by the limited amount of tumor tissue quantity and the poor quality of biopsies available. Both patients P23 and P26 had a partial response to crizotinib and next-generation ALK-inhibitors. Despite the small percentage of *ALK*-rearranged cells detected in the tumor biopsy, these clinical responses may further argue in favor of *ALK*-positivity in patients P23 and P26.

e The biopsy was negative by FISH but positive by IHC.

**Supplementary Table 2.** Descriptive Statistics of Numbers of Circulating Tumor Cells in *Anaplastic Lymphoma Kinase* (*ALK*)-Rearranged Patients at Baseline and Under Crizotinib Therapy

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **CTC detected by FA-FISH** a | | | | | | |  | **CTC counts by CellSearch** b | | |
|  | ***ALK*-rearranged CTCs** | | |  | ***ALK*-CNG CTCs** | | |  |  |  |  |
|  | **Baseline**  **(N=39)** | **Under crizotinib**  **(N=29)** | **Dynamic change**  **(N=29)** |  | **Baseline**  **(N=39)** | **Under crizotinib**  **(N=29)** | **Dynamic change**  **(N=29)** |  | **Baseline**  **(N=38)** | **Under crizotinib**  **(N=29)** | **Dynamic change**  **(N=29)** |
| **Mean** | 18.90 | 19.19 | 0.41 |  | 16.03 | 24.95 | 11.50 |  | 21.08 | 24.66 | -2.00 |
| **Median** | 14.00 | 13.00 | 2.00 |  | 12.00 | 15.00 | 2.00 |  | 0.00 | 0.00 | 0.00 |
| **Standard Deviation** | 19.01 | 16.02 | 18.40 |  | 13.02 | 33.25 | 34.04 |  | 115.36 | 102.02 | 37.17 |
| **Minimum** | 1.00 | 3.00 | -64.00 |  | 1.00 | 2.00 | -18.00 |  | 0.00 | 0.00 | -169.00 |
| **Maximum** | 119.00 | 60.00 | 43.00 |  | 53.00 | 177.00 | 169.00 |  | 713.00 | 544.00 | 89.00 |

Abbreviations: *ALK*, anaplastic lymphoma kinase; CNG, copy number gain; CTC, circulating tumor cell; FA-FISH, filter-adapted fluorescent *in situ* hybridization.

a Three ISET spots were analyzed by combined IF and FA-FISH. Results are expressed as the numbers of *ALK*-rearranged CTC and CTC with gain of *ALK* copies per 3 mL of blood.

b CTC count by CellSearch per 7.5 mL of blood.

**Supplementary Table 3.** Levels of Significant Association between Circulating Tumor Cells Subsets and Clinical Parameters at Baseline

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  | **CTC subsets** | | |
| **Clinical Parameters** | **No. of Patients** |  | ***ALK-*rearranged CTCs** |  | ***ALK*-CNG CTCs** |
| Age at baseline (y/o), ≥55 *v* <55 | 39 |  | 0.162 |  | 0.086 |
| Smoking status (PY), ≥15 *v* <15 | 39 |  | 0.121 |  | 0.002 |
| Number of previous treatment, ≥2 *v* 1 | 39 |  | 0.226 |  | 0.855 |
| ECOG PS at baseline, ≥2 *v* <2 | 39 |  | 0.424 |  | 0.495 |
| Number of metastatic sites, ≥2 *v* 1 | 39 |  | 0.381 |  | 0.621 |
| Brain metastases at baseline, present *v* absent | 39 |  | 0.281 |  | 0.731 |
| Liver metastases at baseline, present *v* absent | 39 |  | 0.111 |  | 0.251 |
| Level of lymphocytes at baseline (/L), <1.106/L *v* ≥1.106/L | 30 |  | 0.481 |  | 0.597 |

Abbreviations: *ALK*, anaplastic lymphoma kinase; CNG, copy number gain; ECOG, eastern cooperative oncology group; PS, performance status; PY, pack-year; y/o, years old.

**Supplementary Table 4.** Successive Treatment Lines Received Post-Crizotinib

|  |  |
| --- | --- |
| Patients | Successive treatment lines received post-crizotinib |
| P1 | No treatment |
| P2 | HSP90 inhibitor then rechallenge with Crizotinib |
| P3 | No treatment |
| P4 | No treatment |
| P5 | Lost after Crizotinib |
| P6 | HSP90 inhibitor, Bevacizumab, Erlotinib, Ceritinib |
| P7 | No treatment |
| P8 | No treatment |
| P9 | Crizotinib on going |
| P10 | Lost after Crizotinib |
| P11 | Paclitaxel/Bevacizumab |
| P12 | HSP90 inhibitor with crizotinib, Ceritinib, Carboplatin/Pemetrexed/Bevacizumab, rechallenge with Crizotinib, Lorlatinib |
| P13 | No treatment |
| P14 | Ceritinib, Lorlatinib |
| P15 | Paclitaxel/Bevacizumab, Erlotinib |
| P16 | Lorlatinib |
| P17 | Ceritinib |
| P18 | No treatment |
| P20 | Crizotinib on going |
| P21 | Crizotinib on going |
| P22 | No treatment |
| P23 | HSP90 inhibitor with crizotinib, Ceritinib, Paclitaxel/Carboplatin, Pemetrexed/Carboplatin, Lorlatinib, rechallenge with Crizotinib |
| P24 | No treatment |
| P25 | HSP90 inhibitor with crizotinib |
| P26 | MET/AXL/FGFR inhibitor, Paclitaxel/Carboplatin, Ceritinib, Paclitaxel/Bevacizumab, Pemetrexed, Pemetrexed/Bevacizumab |
| P27 | Carboplatin/Paclitaxel |
| P28 | Paclitaxel/Bevacizumab |
| P29 | Paclitaxel/Bevacizumab |
| P30 | Crizotinib on going |
| P31 | Lost after Crizotinib |
| P32 | Ceritinib |
| P33 | Docetaxel, Ceritinib, Pemetrexed |
| P34 | Crizotinib on going |
| P35 | Crizotinib on going |
| P36 | Ceritinib |
| P37 | Ceritinib, Alectinib |
| P38 | No treatment |
| P39 | Crizotinib on going |
| P40 | No treatment |