Module 3:
Pathway and Drug Development
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Introduction to Oncomine™

Prerequisites: Module 1: Introduction to Oncomine, Module 2: Introduction to Oncomine Concepts

Training Setup Required:

- Internet access
- Internet Explorer 6.0 (at minimum, Service Pack 3 is strongly recommended), or higher
- Firefox 2 and higher is supported for PC and Mac OS X users
- Java script must be enabled
- Oncomine Login
Welcome to Oncomine 4.3 Training!

This training module is designed to introduce you to the content, pre-computed analyses, and modes of searching the Oncomine cancer profiling database.

By the end of this module, you should be familiar with:

- Using a pre-defined sensitivity signature as a Primary Concept
- Compare gene overlap between Concepts using the Concept Comparison
- Exporting a gene list and uploading it back into Oncomine as a new and unique Concept
- In a subset of cancers, discover a additional sensitivities to Dasatinib
Training Manual Conventions

1. Style Conventions

**Bold** Keyboard-entered text *(MYC)*

**Bold and Italic** Command series *(All > Tumor vs. Normal > Prostate)*

2. Oncomine Notes

Notes appear throughout this manual to provide general information designed to make Oncomine easier to use, or to point out additional capabilities. These notes are easy to distinguish for future reference:

**Note:** In the Huang analysis, breast cancer cell lines were used to identify a Dasatinib sensitivity signature, while the Wang analysis uses prostate cancer cell lines. The Sos CellLine is a DNA copy number dataset.

3. Oncomine Images

This manual provides many images from the Oncomine program to guide you through the training process. Look for the areas highlighted in red to help connect what is being discussed in the manual with what you should see on your computer screen:
1.1 Getting Started

Registration

Users need to be registered for Oncomine 4.

Once logged in, the screen shows the main menu for Oncomine, with drop-down menus at the top, latest content statistics, and search boxes. This is the starting point for any analysis in Oncomine.

- Begin analysis in Oncomine with
- Validating a Dasatinib signature (1.2.1)
- Making a unique Concept (1.2.2)
- Identifying biomarkers (1.2.3)
1.2 Identifying a Dasatinib sensitive cancer signature

1.2.1 Identifying and validating a Dasatinib Signature

Here we will concentrate on finding a Dasatinib sensitive signature, and validate this concept based on previous knowledge.

We are interested in the therapeutic effect of the multi-tyrosine kinase inhibitor Dasatinib, particularly in the treatment of leukemia. Does Oncomine have data on this compound to help us understand the role Dasatinib plays in treating this disease? Can Oncomine validate what is known about this drug and its approved indication for Chronic Myelogenous Leukemia (CML)? Importantly, might Dasatinib be utilized in treating other cancer types, perhaps solid tumors?

1. In Oncomine, you can find a Dasatinib sensitivity signature by:
   a. Using the Filter Tree, select Analysis Type > Differential Analysis > Pathway and Drug Analysis > Drug Sensitivity Analysis > Targeted Therapy Analysis > Dasatinib Sensitivity Analysis.
   b. Using the Search Box: type ‘Dasatinib’ into the Search Box, and select ‘Dasatinib Sensitivity Analysis (Analysis Type)’.


   ![Image](image.png)

   Note: In the Huang analysis, breast cancer cell lines were used to identify a Dasatinib sensitivity signature, while the Wang analysis uses prostate cancer cell lines. The Sos CellLine is a DNA copy number dataset.

3. With the ‘Dasatinib Sensitive-Breast Cancer Cell Line’ from Huang selected, click on the arrow next to ‘+ PRIMARY CONCEPT,’ and select Top 5% over-expressed.

4. When prompted, please select ‘Yes, remove all filters’.
This will return a Concept Summary table, similar to what you see when performing a gene search. Here, however, concepts that associate with the primary concept ‘Dasatinib sensitivity’ are displayed.

**Note:** We provide default thresholds for the concept analysis association results. These may be made more or less stringent by selecting values from the pull down menus. In addition, you can choose to view results for mRNA expression or DNA (copy number) analyses, or both.

Dasatinib is an effective agent for the treatment of patients with imatinib-refractory chronic myelogenous leukemia. Additionally, published studies have linked Dasatinib sensitivity with ‘basal’ breast cancer, particularly with the ‘triple negative’ subtype.

Does the Huang Dasatinib sensitivity signature we selected correlate with these disease phenotypes?

Using the Concept Summary view we can easily link to results that may validate our signature.
5. First, choose the Literature-defined Concepts link by selecting the 43 in the available Literature-defined Concepts field (see above figure).

The most significant association is with basal breast cancer, a known association.

6. Return to the Concept Summary View by clicking the browser’s back button or by selecting the Other Views drop down menu. Next, from the Concept Summary view select the red 9 from the intersection of the Breast Cancer and Molecular Subtype: Biomarker columns.

The molecular biomarker associations are with triple-negative breast cancer analyses from multiple independent studies, including both cell line and primary tumor datasets.

Quite quickly we see validation of the known association with basal breast cancer, and in particular with the triple-negative subtype.
To look for associations with BCR-ABL leukemia:

1. Remove Analysis Type, Cancer Type, & Concept Type from filter using red “x” next to each OR Click the browser’s back button to reset filters leaving “Concept: Dasatinib Sensitive” in the filter, then select under **Analysis Type > Differential Analysis > Cancer Subtype Analysis > Molecular Subtype Analysis > Mutation Analysis**, select **Translocation** OR simply type “transloc” in the search box and choose Translocation (Analysis type) from the auto prompt list.

In the list of Associated Concepts you see a significant association with a BCR-ABL gene fusion analysis from the Ross Leukemia study (Top 5% over-expressed, P-value of 4.11E-14, odds ratio of 2.8), and another from the Yeoh Leukemia study (P-value 3.32E-6, odds ratio 2.3).

To view the association between the Dasatinib sensitivity signature and the BCR-ABL translocation in the Ross leukemia study, first select the Ross study as your associated concept and then select the icon next to ‘Associated Concept’ in the Results pane.

**Note:** This link to a heat map view is available for all Oncomine –derived analyses.
This will launch a heat map of the Dasatinib Sensitive genes imposed on the Ross Leukemia study. Note that the Dasatinib Sensitive genes associate strongly with BCR-ABL leukemia, and not with other rearrangement-dependent leukemia.

In fact, if you toggle back to the concepts view, you will see that the Dasatinib Sensitive genes strongly associate with genes that are expressed at low levels (▼) in MLL-dependent leukemia!

This supports the approved indication for the use of Dasatinib in imatinib-refractory BCR-ABL leukemia, and not with other chromosomal-aberrant leukemias.

2. Remove the Translocation filter by clicking on the appropriate red ‘X’ in the Filter Box, and add the **Pathway and Drug Analysis** filter under the Analysis Type ▶ Differential Analysis branch.
Among the top Associated Concepts is the other Dasatinib sensitivity signature from the Wang CellLine, a significant association displaying a P-value of 1.09E-54 and an odds ratio of 4.8.

The Huang and Wang cell lines are of different histologies (breast vs. prostate), suggesting that Dasatinib may be effective in treating both tumor types.

**Note:** Interestingly, the top two associations are sensitivity to MAPK kinase pathway inhibitors: PD0325901 (a MEK inhibitor), and erlotinib (Tarceva, an EGFR inhibitor).
Current findings:
At this point in our interrogation, we have quickly found known associations with basal/triple-negative breast cancer and with the approved indication for BCR-ABL leukemia. Moreover, Dasatinib sensitivity associates uniquely with BCR-ABL leukemia, i.e. does not strongly associate with other translocation-dependent leukemias. Additionally, Dasatinib strongly associates with multiple MAPK kinase pathway inhibitors.

Additional questions:
What general cancer types exhibit association with Dasatinib sensitivity?

3. Remove the Pathway and Drug Analysis filter and add the Cancer vs. Cancer Analysis filter.

As you can see, there are a variety of associated cancer types, including lung, brain, bladder, kidney and breast. In addition, there are over-expression and under-expression signatures. Because we are interested in genes that are over-expressed, i.e. with genes that may respond to Dasatinib, we can add an over-expression filter to narrow the results further.

4. Under Concept Filters > Concept Type > Oncomine Gene Expression Signatures, select Over-expression (Oncomine Concepts) OR simply type “over” in the search box and choose Over-expression (Oncomine Concepts) from the auto prompt list.

While you see many of the same analyses as before, the addition of the Over-expression signature limits the results to only over-expressed Oncomine Gene Signatures. You can scroll through this list to identify additional cancer types/subtypes that might respond to Dasatinib treatment.
Note: You can also look at Cancer vs. Normal comparisons (remove Cancer vs. Cancer from the filter and add Cancer vs. Normal Analysis) to identify tumor types that associate with Dasatinib sensitivity relative to normal tissue.

Summary

We began our investigation by validating a pre-defined Dasatinib sensitivity signature from the Huang CellLine analysis. After making the Top 5% over-expressed genes a Primary Concept, we began our validation of known associations with the Dasatinib Sensitivity signature.

We first investigated the association with basal/triple-negative breast cancer and found strong associations.

As Dasatinib is known to work on the BCR/ABL gene fusion in CML patients, we added a Translocation filter. Two different BCR/ABL analyses, the Ross Study and the Yeoh study, exhibited significant associations with the sensitivity signature.

After removing the Translocation filter and adding the Pathway and Drug Analysis filter, one of the most significant Associated Concepts was the Dasatinib sensitivity signature from the Wang Prostate Cancer CellLine analysis, with a P-value of 1.09E-54 and odds ratio of 4.8

Confirming that this Dasatinib signature associates with known results boosts confidence that this Concept indeed represents a Dasatinib sensitivity signature, which will then allow us to explore additional associations.

We also used the Cancer vs. Cancer Analysis and Over-expression filter to identify additional Cancer types that may benefit from treatment with Dasatinib. There were several over-expression signatures from lung, brain, bladder, kidney and breast analyses.

1.2.2 Making a unique Dasatinib sensitivity Concept and uploading it into Oncomine

Now that we have validated the Huang Dasatinib sensitivity signature, we might want to examine the overlap between the two Dasatinib sensitivity signatures and make our own unique Concept from the list of overlapping genes. This new Concept can be uploaded into Oncomine for use in future analyses and represents a more generalized Dasatinib sensitivity signature (validated across Prostate & Breast) that may be more effective in analysis of other cancer types.
1. In the Filter Box, remove the Cancer vs. Cancer Analysis filter, leaving the Over-expression filter in place.

2. In the Associated Concepts results pane, find the ‘Dasatinib Sensitive - Prostate Cancer Cell Line - Top 10% over-expressed (Wang CellLine)’ Associated Concept.

3. Click on the box to the left of the Associated Concept and select Compare at the top of the Associated Concepts tab.

This image will appear in the Visualize pane, a list of genes common to both the Primary Concept (Huang Breast Cancer Cell Line) and our selected Associated Concept (Wang Prostate Cancer Cell Line), i.e. a consensus of genes sensitive to Dasatinib across the two selected studies.

From this consensus we will create a new list, which we can use to further validate known Dasatinib associations with leukemia, and potentially identify a role for Dasatinib in treating previously unidentified subsets of cancer.

4. Click on the arrow next to Export, and select Excel-Gene List.

Once you have opened the Excel file, the spreadsheet will look like the one below.
5. Save the gene list as a text (.txt) file. This can be done by copying and pasting the gene list into a text editor, e.g. Notepad. Save this list to a convenient place. In our example, we will call the list Huang and Wang – Dasatinib Sensitivity. One could also simply delete all data except the gene symbol list in the excel spreadsheet and then save as .csv or .txt file.

6. Once you have made the text file, return to Oncomine. In the upper right hand corner click on tools, then select Upload Custom Concept.

7. A new window will pop up, asking for (i) the name of the concept (let's use Huang and Wang), (ii) the file to be uploaded (our saved Huang and Wang – Dasatinib Sensitivity .txt file), and (iii) the Null Set.

There are many Null Sets available. In our example, the two experiments used different microarrays; the Huang analysis used the Human Genome U133A array, and the Wang study used the Human Genome U133A 2.0 array. Because the gene expression for these two datasets was measured on different arrays,
we will use the ‘All Entrez Gene IDs’ as the Null Set. This Null Set will include all of the genes used in either platform.

**Note:** A Concept is a list of biologically relevant genes. You can create your own Concept(s) using any list of genes, whether they were derived from a microarray experiment, or simply a list of, for example, potential kinase targets.

The list must consist of approved HUGO gene names.

8. After Naming the Concept, uploading the appropriate .txt file, and selecting the Null Set, click on Validate.

The validation will let you know if the terms in the uploaded Concept were accepted. In this case, two of our terms ‘10-Sep’ and ‘6-Sep’ were not recognized as HUGO gene symbols.

Despite the two unrecognized terms, 196 genes were successfully validated. To continue, select Upload.
The final window will display the information related to the newly uploaded Concept: Concept Name, the Text File from which it was created, Category and Null Set. It also gives you the option to view our Huang and Wang gene list immediately as a Primary Concept.

9. Click on ‘Select [Huang and Wang] as primary concept now’.

![Upload My Concept](image)

10. One can now explore associated concepts as before using this newly defined Dasatinib sensitivity signature common to Breast and Prostate tissue.

**Summary**

Using Concept Comparison, we compared our Primary Concept with a related Associated Concept. The resulting list of genes represents those in common to both Concepts.

**Note:** You can choose as many Concepts as you like for comparison. The genes found in common across all chosen concepts will appear as green boxes in the resulting display.

We created this list of genes to represent a general ‘Dasatinib sensitivity’ signature. After exporting the list, we uploaded it into Oncomine as a new unique Concept.

Finally, we made it our Primary Concept for association analysis and are ready to continue with our investigation.
1.2.3 Identifying biomarkers in subsets of cancer that indicate a sensitivity to Dasatinib

Now that we have uploaded our unique Concept into Oncomine, can we find important biology associated with Dasatinib sensitivity? Are there other cancer types that show potential sensitivity?

Upon selecting our Primary Concept, an association analysis is performed and a list of Associated Concepts is created. The results are seen in a Concept Summary matrix, a global view of significantly associated Concepts.

![Associated Concept Summary](image)

We could look for underlying biology by selecting the any of the fields found in the ‘Other (Non-Oncomine) Concept Summary field from the bottom panel. We did this earlier (Section 1.2.1) when we looked at Literature-defined Concepts, showing a strong association between the Dasatinib sensitivity signature and basal breast cancer.

However, let’s look for disease associations beyond what we have already discovered.

From the Summary table, we can see recurrent associations in the Cancer vs. Normal column.

11. Select the red 14 next to Brain and CNS Cancer.
The top associations are with Glioblastoma, although other brain cancer subtypes also appear. Scrolling through the list will provide an idea of associated brain tumor subtypes.

12. From the results list, select the boxes next to the four Glioblastoma vs. Normal analyses: the Sun, Lee, Bredel, and Liang studies. Select Compare from the top of the Associated Concepts panel.

There are a number of genes that are common to the five Associated Concepts. Among those, you see EGFR (red arrow) and TGFβ1. These two genes (along with others you identify and confirm with further study) may act as biomarkers that could define a subset of glioblastomas that are treatable with Dasatinib.
Dasatinib has been approved for treatment of imatinib-refractory BCR/ABL leukemia. To see if Dasatinib is affecting the same pathway in both Glioblastoma and BCR-ABL leukemia, we can compare genes that are significantly expressed in the two cancer types.


14. From the filter tree: **Analysis Type > Differential Analysis > Cancer Subtype Analysis > Molecular Subtype Analysis > Mutation Analysis > Translocation.**

15. Check the boxes next to the two BCR-ABL gene fusion analyses, Ross Leukemia and Yeoh Leukemia. Select Compare.

Oncomine remembers/retains the previously checked boxes for the Glioblastoma analyses. We have now added the BCR-ABL leukemia analyses for comparison.

Although our Dasatinib sensitivity signature does strongly associate with BCR-ABL leukemia and glioblastoma, it appears that these associations are with subsets of the signature that do not significantly overlap with both diseases.

Perhaps this is not surprising, since BCR-ABL leukemia is driven by the ABL tyrosine kinase (a target of imatinib), while glioblastoma is often associated with mutant EGFR (and other genetic abnormalities).

This suggests that Dasatinib, a somewhat promiscuous tyrosine kinase inhibitor, may affect different pathways in the two diseases, i.e. the gene expression patterns between the two cancers are largely non-overlapping.
**Note:** Looking at the concept Summary matrix (Section 1.2.3, page 19), significant associations are also seen with Head and Neck, Kidney, Liver, and Pancreatic Cancer. We will not explore these here, but this does suggest additional cancer types that may benefit from Dasatinib treatment, which can easily be explored.

### Overall Summary

After uploading a Dasatinib Sensitivity Concept, we attempted to find important known as well as undiscovered biological associations in the Oncomine data.

The addition of the Literature-defined Concepts filter showed an association with the basal subtype of breast cancer. Applying a biomarkers filter, we saw strong associations with triple-negative breast cancer occurring in multiple independent studies, a known association.

Additional validation was observed by significant overlap with BCR-ABL leukemia, but not with other leukemia subtypes.

An association was also seen with another Dasatinib sensitivity study, although derived from a different cell type.

We next demonstrated how to upload unique ‘Concepts’ for association analysis.

Using the Concept Summary view, we could see additional cancer types and analyses that exhibit strong associations with Dasatinib sensitivity. We explored one of these, brain cancer.

Many of the top associated Concepts were Glioblastoma vs. Normal analyses. By checking the boxes next to 4 independent analyses, we made a Concept Comparison, which could be seen in the Visualize pane.

As seen earlier, this list represents the overlap of genes between the Primary Concept and the subsequent Associated Concepts. EGFR and TGFβ1 are common to all of the Concepts. These two genes could represent biomarkers that are present in a subset of glioblastomas that might be sensitive to Dasatinib.

To see if Dasatinib affects the same pathway in glioblastoma as it does in BCR-ABL leukemia, we added the appropriate filters to define a new Concept Comparison.

The absence of gene expression of a number of genes in the leukemia analyses, particularly EGFR and TGFβ1, was clearly seen. The gene expression pattern in the Concept Comparison suggests that Dasatinib is perhaps working on different pathways in the two diseases.
1.2.4 Oncomine Support

support@compendiabio.com

1-866-369-5070

(M–F, 8:30 A.M.–5:30 P.M. ET)

Notes: